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WITH THE ASSISTANCE OF

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THE INFLUENCE OF IRON SUPPLY ON TOXIC EFFECTS OF MANGANESE, MOLYBDENUM AND VANADIUM ON SOYBEAN, PEAS AND FLAX

By KATHERINE WARINGTON

Rothamsted Experimental Station, Harpenden, Herts

(With Plates 1 and 2, and 1 Text-figure)

The investigations were carried out in nutrient solution with iron as ferric citrate and nitrogen in the form of nitrate.

Addition of 2·5 p.p.m. vanadium to plants in which iron chlorosis was already established, either by a lack of iron or by excess manganese, failed to counteract the condition, and caused toxic symptoms.

Reduction of the standard iron supply to $\frac{1}{2}$ or $\frac{1}{3}$ accentuated the toxicity of 2·5 or 5 p.p.m. V to soybean and flax, but a similar reduction in phosphorus had no influence. Toxicity to peas, however, was increased when the phosphorus was reduced to $\frac{1}{10}$, provided the iron level was high (20 p.p.m. Fe).

Raising the iron supply to 20 or 30 p.p.m. Fe counteracted the toxicity of manganese (10 p.p.m.), molybdenum (40 p.p.m.) and vanadium (2·5 p.p.m.), but the result was less marked when these three elements were combined. Iron supplied in successive, small doses proved less efficient in overcoming molybdenum or vanadium, but not manganese excess, than the same amount of iron supplied in fewer and larger quantities. Varying the iron supply had little effect when the concentration of the three elements was low. When increased iron supply had reduced the chlorosis caused by high manganese or vanadium, it also reduced the manganese and vanadium contents of the shoot (p.p.m./d.m.), but the molybdenum content was only lowered by high iron when given in non-toxic concentration (0·1 p.p.m. Mo) combined with excess manganese.

The iron content of the shoot (%/d.m.) was scarcely affected by the amount of iron supplied, but was generally reduced by high concentrations of manganese, molybdenum or vanadium.

Results regarding the effect of vanadium on the phosphorus content of the shoot were conflicting, and differences occurred only when the iron supply was low. Here the phosphorus content of soybean and peas was generally reduced, while that of flax was increased.

Yield data for soybean and flax indicated an interaction between manganese with both molybdenum and vanadium if the iron supply was low, but none between molybdenum and vanadium. The effect of all three metals was additive in respect to iron.

I. Introduction

In some previous experiments concerned with the inter-relationships between manganese, molybdenum and vanadium in the nutrition of soybean and flax (Warington, 1951), it had been shown that within the range of concentrations tested, molybdenum and vanadium exerted contrasting effects on the chlorosis induced by excess manganese. Whereas molybdenum accentuated the symptoms, as found also by Hewitt (1949), high vanadium caused at least a temporary deepening of colour in the shoot, though chlorosis usually followed later. It was not clear, however, whether this subsequent chlorosis was due to the high level of vanadium (in which case the element fell into line with many others including manganese), or was caused by a reduced absorption of iron owing to the accompanying restriction of the root system. Analysis had shown an increased total iron but reduced phosphorus content of flax shoots treated with high vanadium, facts which fitted in with the appearance of the plants and to which attention had already been drawn. As will be seen later, these analytical results were not always confirmed and varied with the crop grown. The present investigations were primarily designed to test the interactions between vanadium and manganese, and between vanadium, iron and phosphorus in greater detail; subsequently, the effect of iron supply on vanadium toxicity was explored. Parallel tests were also made with excess manganese and molybdenum for comparison with work done in 1950.

The experiments fall into three groups, viz. interactions between:

A. V, Mn and Fe (soybean and flax).
B. V, Fe and P, V and Fe (soybean, flax and peas).
C. V, Mn, Mo and Fe (soybean and flax).

In group *A*, the interaction between the micronutrients under conditions of iron deficiency was studied, whereas in groups *B* and *C*, their effects were compared when the iron supply ranged from low to plentiful.

II. GENERAL TECHNIQUE AND PROCEDURE

The van der Crone nutrient solution used in the 1950 work, was clearly unsuitable for accurate measurement of the iron and phosphorus supplied, and a Rothamsted solution,* containing soluble phosphates and ferric citrate was substituted. When a reduced rate of phosphorus was required, compensation for the loss of potassium was made with potassium chloride. The iron supply was varied according to whether deficiency, adequate or excess levels were required. The rates selected are cited under the appropriate sections, the term 'standard' indicating a routine control level. Iron was supplied only when fresh nutrients were given, any modification of the rate which seemed desirable being deferred to a subsequent experiment. The effect of frequency of the iron application was tested on two occasions.

All solutions were started at a pH of 4·6 to ensure good initial availability of the iron; N/10-HCl or NaOH were added as needed. Table 10 records changes in pH value of solutions during growth.

Boron and copper, together with manganese and molybdenum when not included in the experimental treatment, were always added to the basal nutrients. If manganese and molybdenum formed the subject of investigation, they were supplied at two rates, one as a control to ensure against deficiency and a higher rate at toxic level. Vanadium was supplied in a greater number of concentrations, including intermediate levels. Chemical equivalence had not been found a convenient form of comparison, so the concentrations selected were based on previous experience. Expressed as p.p.m., the usual low 'control' rates were as follows: manganese as $MnSO_4.4H_2O$, 1·0; molybdenum as $Na_2MoO_4.2H_2O$, 0·1; vanadium as $VOCl_2$ 0·1; boron as H_3BO_3, 1·0; copper as $CuSO_4.5H_2O$, 0·05. The higher rates are quoted under the appropriate sections.

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* KNO_3, 1·0 g./l.; $MgSO_4 . 7H_2O$, 0·5 g./l.; KH_2PO_4, 0·45 g./l.; K_2HPO_4, 0·054 g./l.; $CaSO_4 . 2H_2O$, 0·5 g./l.; ferric citrate, various; micronutrients, see text.

Plants were usually set up singly in 400 or 600 ml. bottles, five replicates per treatment or more if material was required for analysis. The cultures were not aerated. The varieties grown were flax (Liral Prince), soybean (Swedish 201–14–18) and peas (Kelvedon Wonder). Colour was judged by eye. Scoring was from 1 to 10, 5 denoting chlorosis and 7 a 'normal' green plant. The distinction between 5 and 6 was specially sharp, while 8 or 9 indicated the appearance of the blue-green colour associated with excess vanadium. The white chlorosis of the high manganese treatments was clearly more damaging than the golden yellow of high molybdenum and was given a lower figure.

The mean yields varied so widely with treatment that separate standard errors were computed for each. Only significant differences are discussed in the text. The data in Table 8 were analysed after conversion to logarithms.

III. EXPERIMENTAL DATA AND RESULTS

A. *Interaction between vanadium, manganese and iron. Comparison between the effect of vanadium on the iron deficiency symptoms caused either by manganese excess or by direct shortage of iron*

Excess manganese, deficient iron or high vanadium were all known to produce chlorosis, but only high vanadium caused a much restricted and abnormal root system. Since vanadium initially improved leaf colour, the ultimate chlorotic condition might presumably be an indirect effect, due to reduced iron intake of the abnormal roots. Soybean (26 April–16 July) and flax (4 April–7 June) were treated in 1951 with all combinations of (*a*) 1 and 20 p.p.m. Mn, (*b*) 5 and 10 p.p.m. Fe, (*c*) 0·5 and 5·0 p.p.m. V.* The manganese and iron treatments were given from the start, but the applications of vanadium to soybean were delayed for 29 days, and to flax for 40 days to permit root development.

The high level of manganese readily produced chlorosis, but the lower concentration of iron failed to give deficiency symptoms in the soybean, while the higher one did not prevent temporary chlorosis in flax. In the low manganese group where the plants were healthy, the addition of 0·5 p.p.m. V had no effect, but the 5 p.p.m. rate was injurious at both levels of iron. This high vanadium did not always cause apical chlorosis, but leaves at the base of the shoots were yellowed in soybean and scorched at the tips and edges of flax. With plants already dwarfed and chlorotic from manganese excess, specific vanadium injury was less easy to detect, and neither rate seemed to have much influence on dry weight or appearance of soybean, though 5 p.p.m. V caused a significant drop in total dry weight of flax (Table 1). Root injury developed on both crops following the addition of high vanadium, irrespective of the level of manganese or iron supplied. The symptoms were a blue-grey discoloration, swelling, splitting and corkiness, the two latter features being unexpectedly more noticeable in the presence of low than of high manganese. Thus vanadium, at the rates tested, seemed incapable of counteracting the symptoms of manganese excess once they were well established, and the chlorosis induced by the vanadium was probably not a result of the restricted root system, though the evidence is admittedly inconclusive.

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* For convenience of comparison with 1950 experiments it may be noted that 5 p.p.m. V ≡ 10 p.p.m. Mo approx.

Table 1. *Dry weight of plants grown with different combinations of vanadium, manganese and iron.* (*Mean of* 5 *plants.*) 1951

	Treatment (p.p.m.)		Soybean			Flax		
	Mn	(V)	Shoot (g.)	Root (g.)	Total (g.)	Shoot (g.)	Root (g.)	Total (g.)
Standard Fe*	Low (1·0)	0·5	8·75	2·19	10·94	2·02	0·54	2·56
		5·0	4·56	1·35	5·91	1·07	0·26	1·33
	High (20)	0·5	1·36	0·48	1·84	0·89	0·29	1·18
		5·0	1·26	0·45	1·71	0·71	0·20	0·91
½ Fe	Low (1·0)	0·5	8·99	2·48	11·47	1·76	0·48	2·24
		5·0	4·89	1·36	6·25	0·89	0·21	1·10
	High (20)	0·5	1·77	0·52	2·29	0·72	0·24	0·96
		5·0	1·26	0·43	1·69	0·51	0·17	0·68
			Differences in total dry weight significant, except between levels of V when Mn high, or between levels of Fe			Differences in total dry weight significant, except between levels of Fe when Mn low		

* 10 p.p.m. Fe.

B. *Interactions between vanadium, iron and phosphorus. Influence of iron and phosphorus supply on the toxicity of vanadium*

To determine whether the characteristic appearance of plants grown with high vanadium was associated with disturbances in their iron and phosphorus nutrition, four tests were made in 1951, two with flax (19 March–4 June, 27 June–22 August) and one each with soybean (27 June–1 September) and peas (25 August–25 October). The same design was used in all experiments, but the rates of the elements supplied were varied as experience was gained. Phosphorus was first halved, later decreased to one-third and finally to one-tenth of the standard rate, and even then visual symptoms of deficiency were only slight. 10 p.p.m. Fe served as the standard rate for flax and soybean; and one-half or one-third this amount produced the desired deficiency symptoms. For peas, the standard rate was 5 p.p.m. Fe and the concentrations ranged from 1·25 to 20 p.p.m. For vanadium, the lower limit of 0·05 p.p.m. was replaced by 0·1 p.p.m. V after the first test. 5 p.p.m. V served as the upper limit throughout. The treatments, all combinations of which were tried, are summarized in Table 2.

TABLE 2. *Rates of phosphorus, iron and vanadium supplied in the nutrient solution. 1951 experiments (p.p.m.)*

	Flax I	Flax II and Soybean	Peas
Phosphorus	56 and **112**	37·3 and **112**	11·2 and **112**
Iron	5 and **10**	3·3 and **10**	1·25, **5·0** and 20
Vanadium	0·05, 0·5 and 5·0	0·1, 0·5, 2·5 and 5·0	0·1, 2·5 and 5·0

Standards are given in black type.

Treatments were started as soon as the seedlings were large enough for culture vessels. With flax, this was after 7 or 12 days' growth on waxed muslin stretched over the standard control solution (without vanadium) diluted $\frac{1}{5}$, but soybean and peas were transferred when 5 days old, directly from the washed, sterilized sand in which they had been germinated. In the first set of flax slight chlorosis occurred in the controls receiving standard (10 p.p.m.) Fe, and to avoid possible premature death in the low iron treatments, a uniform addition of 0·25 mg. Fe per plant, followed by two further additions of 0·5 mg. was made. Subsequently, however, iron was given only when the nutrient solutions were renewed, and the importance of a standardized technique for iron administration became abundantly clear as the work progressed.

(*a*) *Soybean and flax*

The symptoms of high vanadium treatment appeared first in the roots, varying from almost complete suppression of laterals to a short, stubby root system or a thickening of the root tips only. Toxicity with 2·5 or 5·0 p.p.m. V was usually evident after about a week, but the degree of the damage depended on the iron, and to a less extent on the phosphorus supplied.* For example, in soybean receiving phosphorus and iron at the standard rates, only 5 p.p.m. V injured the roots at first, whereas 2·5 p.p.m. V was nearly as harmful if the phosphorus and/or iron were reduced to $\frac{1}{3}$. Later the shoot showed similar differential effects, vanadium toxicity being measured by intensity of leaf colour and onset of chlorosis. In flax I, 5 p.p.m. V deepened the leaf colour in all solutions, *except* those with standard P and $\frac{1}{2}$ Fe, where chlorosis appeared almost from the start.

Conversely, the increase in colour was greatest and most prolonged where the iron was potentially at the highest level ($\frac{1}{2}$ P and standard Fe), the effects being intermediate when both nutrients were at the half or standard rate (Text-fig. 1). In the presence of low vanadium (0·05 p.p.m. V) the changes in iron level had little influence on colour. 5 p.p.m. V produced chlorotic symptoms in all solutions, but most readily with standard P and $\frac{1}{3}$Fe, followed closely by the set with $\frac{1}{3}$P and $\frac{1}{3}$Fe. Least chlorosis appeared with the $\frac{1}{3}$P and standard Fe treatment, where iron was presumably most available. 2·5 p.p.m. V produced no chlorosis in soybean when iron was at the standard rate, nor in flax, if the phosphorus was reduced at the same time. Changes in the phosphorus or iron supplied had no apparent influence when vanadium was at still lower rates (0·5 or 0·1 p.p.m.), soybeans remaining a normal colour, while all the flax became slightly chlorotic towards the end of the experiment, suggesting that 10 p.p.m. Fe with only fortnightly renewal was inadequate as a standard for this plant.

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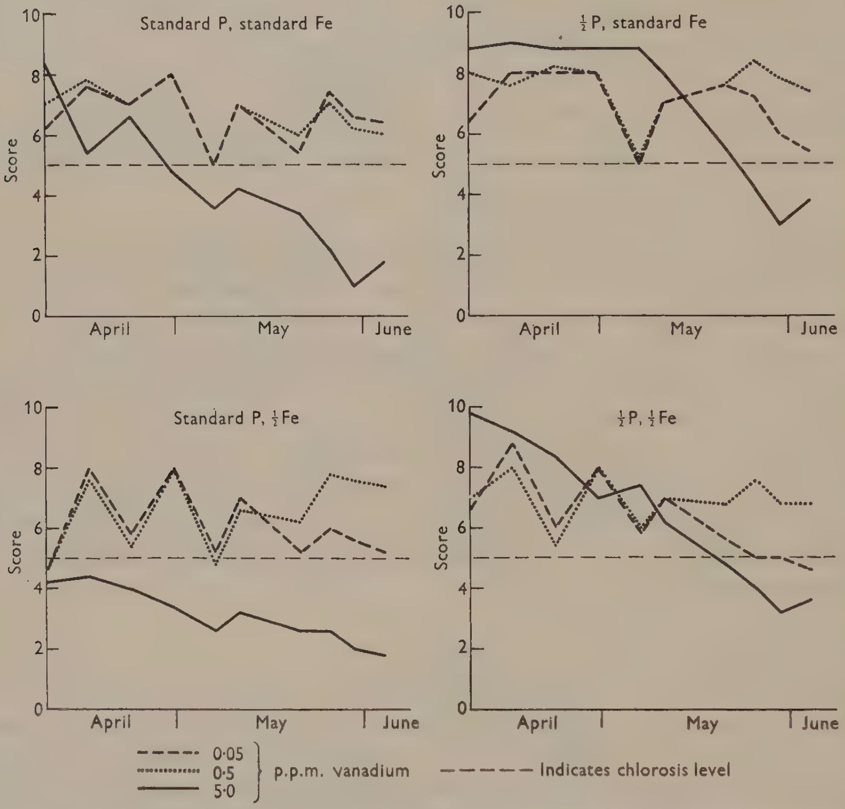
* It was realized that a reduction in the phosphorus content of the nutrient solution would probably increase the availability of the iron, and thus exert an indirect as well as direct influence on the vanadium-iron-phosphorus relationship.



Text-fig. 1. Effect of vanadium on colour of shoot apex in the presence of standard and reduced iron and phosphorus supply. Flax I. 1–10 denotes increasing greenness.

Differences in appearance were reflected in dry weights. In the spring-sown flax, a fall began at 0·5 p.p.m. V, irrespective of the iron or phosphorus treatment, but the degree of reduction with this as with the higher rate, depended on the iron content of the solution, being most marked where the iron supply was low (Table 3). In the two subsequent experiments (Table 4), damage started at 0·5 p.p.m. V only if both the phosphorus and iron supplies were reduced, but 2·5 or 5 p.p.m. V were

TABLE 3. *Dry weight and phosphorus content of flax I* (1951) *grown with different concentrations of vanadium, phosphorus and iron.* (*Mean of* 5 *plants*)

Treatment	V (p.p.m.)	Dry weight Shoot (g.)	Root (g.)	Total (g.)	Shoot (d.m.) (P %)
Standard P, standard Fe*	0·05	3·11	0·74	3·85	0·47
	0·50	2·01	0·53	2·54	0·50
	5·00	0·08	0·03	0·11	0·84
Standard P, ½Fe	0·05	2·67	0·76	3·43	0·54
	0·50	1·49	0·37	1·86	0·54
	5·00	0·02	0·01	0·03	1·34
½P, standard Fe	0·05	4·02	0·92	4·94	0·41
	0·50	2·81	0·54	3·35	0·41
	5·00	0·13	0·03	0·16	0·41
½P, ½Fe	0·05	2·94	0·74	3·68	0·47
	0·50	2·09	0·42	2·51	0·38
	5·00	0·10	0·03	0·13	0·45

* 10 p.p.m. Fe.

TABLE 4. *Dry weight and phosphorus content of plants grown with different concentrations of vanadium, phosphorus and iron.* 1951. (*Mean of* 5 *plants*)

		Flax II. Dry weight				Soybean. Dry weight			
Treatment	V (p.p.m.)	Shoot (g.)	Root (g.)	Total (g.)	Shoot (d.m.) (P %)	Shoot (g.)	Root (g.)	Total (g.)	Shoot (d.m.) (P %)
Standard P, Fe*	0·1	1·43	0·35	1·78	0·64	7·64	1·23	8·87	0·72
	0·5	1·32	0·29	1·61	0·53	5·81	1·09	6·90	0·88
	2·5	0·29	0·06	0·35	0·62	4·00	0·75	4·75	0·72
	5·0	0·10	0·04	0·14	0·65	1·36	0·55	1·91	0·39
Standard P, ⅓Fe	0·1	1·25	0·36	1·61	0·67	5·63	1·23	6·86	0·91
	0·5	0·98	0·23	1·21	0·61	4·89	1·04	5·93	0·84
	2·5	0·06	0·03	0·09	0·95	1·77	0·48	2·25	0·47
	5·0	0·03	0·03	0·06	1·00	0·88	0·32	1·20	0·49
⅓P, standard Fe	0·1	1·48	0·30	1·78	0·46	6·90	1·18	8·08	0·43
	0·5	1·52	0·34	1·86	0·47	9·58	1·53	11·11	0·38
	2·5	0·33	0·06	0·39	0·40	2·80	0·70	3·50	0·48
	5·0	0·10	0·03	0·13	0·33	1·58	0·52	2·10	0·29
⅓P, ⅓Fe	0·1	1·15	0·27	1·42	0·45	7·60	1·36	8·96	0·43
	0·5	0·71	0·14	0·85	0·50	5·46	1·07	6·53	0·59
	2·5	0·10	0·03	0·13	0·42	2·12	0·68	2·80	0·33
	5·0	0·07	0·03	0·10	0·36	0·99	0·38	1·37	0·31

* 10 p.p.m. Fe.

invariably harmful. Not only was vanadium damage lessened by an increase in iron but soybean benefited from 0·5 p.p.m. V in the treatment providing potentially most iron ($\frac{1}{3}$P and standard Fe).

(*b*) *Peas*

In the experiment with peas the iron treatments included a concentration (20 p.p.m. Fe) expected to exceed the normal requirement. As with soybean and flax, the first differences between treatments appeared in the roots. After about a week both 2·5 and 5·0 p.p.m. V were toxic *except* in the presence of standard P and 20 p.p.m. Fe, though 5 p.p.m. V caused some injury with this level of iron if the phosphorus was reduced to $\frac{1}{10}$. Later, some damage with 5 p.p.m. V became general, though it always remained slight where iron and phosphorus were both at the high level. Similar effects appeared in the shoots. No chlorosis developed, due no doubt to the lateness of the season, but all plants receiving 2·5 or 5·0 p.p.m. V showed an intensified green colour for about a fortnight and at harvest were very undersized, *except* where standard P and 20 p.p.m. Fe were supplied (Pl. 1, figs. 1–4). These plants were normal in appearance and their total dry weight not significantly below that of the controls grown with low (0·1 p.p.m.) V (Table 5). Both 2·5 and 5·0 p.p.m. V reduced yield in all other solutions including that providing 20 p.p.m. Fe and low phosphorus. Thus a reduction in iron, and to a less extent the phosphorus content, increased the damage from vanadium, while toxic effects of this element

TABLE 5. *Dry weight and phosphorus content of peas grown with different concentrations of vanadium, phosphorus and iron.* 1951. (*Mean of* 5 *plants*)

		Dry weight			
Treatment	V (p.p.m.)	Shoot (g.)	Root (g.)	Total (g.)	Shoot (d.m.) (P %)
Standard P, 1·25 p.p.m. Fe	0·1	1·69	0·42	2·11	0·63
	2·5	0·69	0·14	0·83	0·46
	5·0	0·46	0·10	0·56	0·48
Standard P, 5 p.p.m. Fe	0·1	1·49	0·36	1·85	0·57
	2·5	0·70	0·15	0·85	0·43
	5·0	0·51	0·14	0·65	0·48
Standard P, 20 p.p.m. Fe	0·1	1·69	0·44	2·13	0·52
	2·5	1·57	0·44	2·01	0·48
	5·0	1·53	0·35	1·88	0·45
$\frac{1}{10}$ P, 1·25 p.p.m. Fe	0·1	1·95	0·55	2·50	0·38
	2·5	0·58	0·12	0·70	0·33
	5·0	0·33	0·07	0·40	0·36
$\frac{1}{10}$ P, 5 p.p.m. Fe	0·1	1·74	0·41	2·15	0·41
	2·5	0·74	0·14	0·88	0·36
	5·0	0·46	0·09	0·55	0·30
$\frac{1}{10}$ P, 20 p.p.m. Fe	0·1	1·53	0·40	1·93	0·29
	2·5	1·11	0·26	1·37	0·29
	5·0	0·70	0·15	0·85	0·26

were almost overcome, provided the level of the iron supplied was sufficiently high. Morris & Pierre (1947), however, found that there was a limit to the effect of iron in counteracting manganese toxicity. This V and Fe interaction was further explored in the two following experiments.

(*c*) *Peas and flax*

Cultures of peas (15 May–8 July) and flax (4 March–16 May) were set up in 1952 with treatments combining two levels of vanadium (0·1 and 2·5 p.p.m. V) with three rates of iron. For peas, 1·25, 5·0 and 20 p.p.m., and for flax, 5·0, 12·0 and 30 p.p.m. Fe were used. All plants received a standard supply of phosphorus.

In the 0·1 p.p.m. V series, all the peas made good growth (Pl. 1, fig. 5). Slight chlorosis developed periodically with the lowest amount of iron, while the root tips were abnormally thickened in the 20 p.p.m. Fe set (see Millikan, 1949). In the 2·5 p.p.m. V group, however, toxicity was severe with 1·25 p.p.m. Fe, reduced with 5 p.p.m. and largely overcome by 20 p.p.m. Fe, the increase in total yield with even the first increment in iron being significant (Pl. 1, fig. 6, and Table 6). The lighter colour of the plants with high iron seen in the photographs, was due to greater maturity. The results with flax were very similar, good growth occurring with all levels of iron in the low (0·1 p.p.m.) V series. Chlorosis developed in the 5 p.p.m. Fe set, but there was no evidence in this series that 30 p.p.m. was excessive (see p. 13). In the presence of high (2·5 p.p.m.) V, severe dwarfing and yellowing occurred if only 5 or 12 p.p.m. Fe was provided, but with 30 p.p.m. Fe, shoot colour merely deteriorated slowly, the plants flowered and the final yield, though below that of the controls, was greatly improved (Pl. 1, fig. 7, and Table 6).

The nutrient solutions containing the higher concentrations of iron showed precipitation even at a pH of 4·6, and loss of iron supply might be expected to increase as the reaction became more alkaline. Iron was normally supplied in a single dose whenever fresh nutrients were given, but it seemed possible that a given quantity of iron might counteract vanadium toxicity better if supplied in a succession of small doses. A preliminary trial with peas showed that a total of 20 mg. Fe given on eighteen separate occasions was *less* efficient in counteracting the effect of 5 p.p.m. V than the same amount given in two doses only (see Twyman, 1951).

Analytical data are discussed in § C (3).

C. *Interactions between vanadium, manganese, molybdenum and iron*

(1) *Influence of iron on the toxicity of manganese, molybdenum, and vanadium alone and in combination*

The influence of iron supply on vanadium toxicity suggested parallel experiments on the toxicity of manganese and molybdenum.

Soybeans were grown (19 April–25 June 1952) with either manganese, molybdenum or vanadium at a high level (10 p.p.m. Mn, 40 p.p.m. Mo, 2·5 p.p.m. V),

TABLE 6. *Dry weight and analyses of plants grown with different combinations of vanadium and iron.* 1952. *Mean of* 10 *cultures* (*low* V), 20 *cultures* (*high* V)

	Low V (0·1 p.p.m.)						High V (2·5 p.p.m.)					
	Dry weight			Shoot (d.m.)			Dry weight			Shoot (d.m.)		
Iron treatment (p.p.m., see text)	Shoot (g.)	Root (g.)	Total (g.)	V (p.p.m.)	Fe (%)	P (%)	Shoot (g.)	Root (g.)	Total (g.)	V (p.p.m.)	Fe (%)	P (%)
						Peas*						
Low	7·97	0·94	8·91	0·31	0·010	0·39	2·79	0·34	3·13	4·49	0·009	0·41
Standard	8·45	0·95	9·40	0·24	0·007	0·39	4·20	0·46	4·66	2·15	0·008	0·37
High	7·42	0·90	8·32	0·15	0·012	0·37	6·43	0·76	7·19	1·90	0·007	0·33
Sign. diff. in total dry wt.	None						1st increment of Fe only					
						Flax†						
Low	4·69	1·32	6·01	0·07	0·003	0·43	0·35	0·15	0·50	7·14	0·003	0·77
Standard	3·70	0·98	4·68	0·06	0·002	0·47	0·39	0·16	0·55	6·64	0·005	0·75
High	6·12	1·50	7·62	0·07	0·002	0·33	2·22	0·52	2·74	3·78	0·004	0·40
Sign. diff. in total dry wt.	All						2nd increment of Fe only					

* Four plants per culture.

† Two plants per culture.

combined with the two remaining elements at the low rate, all three at the low rate serving as control (for low rates see § II). Each combination of elements was tested in the presence of 2·5, 5, 10 and 20 p.p.m. Fe. The sets with the highest level of iron were duplicated, one half receiving ferric citrate only when fresh nutrient solution was supplied, the other being given the same total of iron in small doses on alternate days. Yields and analytical data are given in Table 7.

Controls. Although both the higher rates of iron improved the colour of the shoots, particularly in the young stages, growth and dry weight were little affected. The manner in which the largest quantity of iron was given was also immaterial. All plants flowered and fruited normally.

High manganese. The level of iron supplied proved of considerable importance in this group. Apical chlorosis and brown leaf speckling over the whole shoot developed rapidly with the two lower rates of iron and the plants remained dwarfed and failed to flower. At 10 p.p.m. Fe, apical symptoms were less severe and flowers, but no pods, were formed. There was much leaf curling, symptomatic of manganese excess. In the presence of 20 p.p.m. Fe, no chlorosis developed and pods were produced. Leaf curling was less noticeable than in the 10 p.p.m. Fe set, and the plants began to approach normal. All increments of iron, with the exception of the lowest, gave a significant increase in yield, the method of applying the 20 p.p.m. rate being of no consequence.

High molybdenum. The toxic effects of molybdenum were slower to appear than those of manganese, but the influence of the iron supply on the severity of the symptoms was well marked. With only 2·5 p.p.m. Fe apical chlorosis followed by leaf speckling soon became acute, and flowers were not formed. The golden colour (Warington, 1937) failed to appear, possibly because the concentration of molybdenum was too low. With 5 p.p.m. Fe, the onset of chlorosis was delayed, and plants receiving 10 or 20 p.p.m. Fe were almost normal. Differences in total dry weight were significant for each increment of iron except the last and also for the methods of application, the gradual addition of the ferric citrate proving less effective than the few larger doses.

High vanadium. Increasing the iron supply again markedly reduced vanadium toxicity. With 2·5 or 5 p.p.m. Fe root and shoot injury was severe and no flowers formed. Deepened leaf colour appeared, followed by apical chlorosis, though the dark colour was retained in the lowest leaves: there was no brown speckling. 10 or 20 p.p.m. Fe gave nearly normal plants with well-developed flowers and pods. There was no marked blue-green phase nor chlorosis, i.e. no characteristic symptoms of vanadium poisoning. These higher iron supplies increased dry weight, though not to the level of those of the controls. As in the high-molybdenum group, the gradual addition of iron at the 20 p.p.m. rate was less effective than the few large doses in counteracting damage from vanadium. The higher vanadium content of the shoot under these latter conditions is noteworthy (Table 7).

The general conclusions were: (1) that the toxic effects of high concentrations of

TABLE 7. *Dry weight and analyses of soybean grown with excess manganese, molybdenum or vanadium in the presence of various concentrations of iron.* 1952. (*Mean of 5 plants*)

Iron content of nutrient solution (p.p.m. Fe)	Dry weights (g.)											
	Control			High Mn (10 p.p.m.)			High Mo (40 p.p.m.)			High V (2·5 p.p.m.)		
	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
2·5	8·03	2·40	10·43	0·62	0·21	0·83	1·36	0·42	1·78	0·86	0·42	1·28
5·0	8·02	2·25	10·27	1·11	0·41	1·52	3·94	1·18	5·12	1·20	0·47	1·67
10·0	8·14	2·30	10·44	2·40	0·66	3·06	6·24	2·03	8·27	4·34	1·41	5·75
20·0	8·64	2·49	11·13	5·48	1·54	7·02	6·66	1·94	8·60	7·26	1·84	9·10
20·0 given gradually	8·97	2·24	11·21	5·39	1·26	6·65	4·16	1·25	5·41	5·11	1·59	6·70

S.E. on total dry weight = 0·4304

p.p.m. Fe	Analyses (shoot p.p.m./d.m.)															
	Mn	Mo	V	Fe	Mn	Mo	V	Fe	Mn	Mo	V	Fe	Mn	Mo	V	Fe
5·0	90	9	<0·05	47	1021	34	0·19	28	125	47	<0·05	23	127	9	1·66	25
10·0	82	10	<0·05	50	930	32	0·09	17	94	51	<0·05	33	83	12	0·68	22
20·0	73	9	<0·05	48	556	14	<0·05	29	93	59	<0·05	34	54	11	0·56	20
20·0 given gradually	72	7	<0·05	48	547	12	<0·05	38	124	43	<0·05	29	63	13	0·97	20

manganese and molybdenum as well as vanadium could be mitigated by increasing the iron supply in the nutrient solution; (2) that results might be appreciably altered by a change in the method of supplying the iron; and (3) that although apical chlorosis ultimately developed with excess manganese, molybdenum or vanadium, the elements showed individual differences in the other symptoms induced, e.g. root damage with vanadium and leaf curling with manganese.

Very similar conclusions were reached in two further experiments with soybean (4 July–8 September) and flax (24 July–16 September) in 1952. Different levels of iron in the nutrient solution were tested in the presence of high manganese, molybdenum and vanadium in all combinations as well as alone. The concentrations of these three elements were the same as had been used in the previous experiment. Iron was given as 2·5, 5 and 20 p.p.m. Fe for soybean and 5, 12, 30 p.p.m. Fe for flax. Results are given in Table 8 and Pl. 2. figs. 8–11.

Controls (Pl. 2, fig. 8). In this group soybean was little affected by the concentration of iron. With flax 30 p.p.m. Fe induced deepened shoot colour, stunted apices, aborted flower buds and tip-scorched, prematurely-shed lower leaves. The dry weight, however, was unaffected. Both crops flowered at all levels of iron.

High manganese (Pl. 2, fig. 9). The previous results were confirmed. The highest rate of iron caused much leaf curling in soybean and symptoms suggesting iron excess (see controls) in flax, but it removed the apical chlorosis and lower leaf speckling induced in both crops grown with the two smaller amounts of iron. Improvement in yield, accompanied by flowering, occurred only with the highest level of iron in soybean. Roots were invariably normal.

High molybdenum (Pl. 2, fig. 10). Molybdenum toxicity was again slow to appear, and in flax was confined to visual symptoms, but in both crops the damage was much reduced by increasing the iron supply. 30 p.p.m. Fe prevented the appearance of the golden colour in flax, though Millikan (1949) found that additional iron was ineffective in this respect. The maximum quantity of iron he supplied, however, appears to be lower than that used in the present experiments. In soybean, both lower levels of iron caused marked apical chlorosis and dry-weight reduction, but the toxic effects were clearly offset by 20 p.p.m. Fe, which allowed pod formation. Freedom from speckling on the basal leaves irrespective of the iron supply, and the horizontal position of the leaves where the iron was low, were further features distinguishing this group from that with high manganese. Flax roots showed molybdenum-induced thickening and yellow discoloration with the low iron treatment.

High vanadium (Pl. 2, fig. 11). With the highest rates of iron both crops flowered and shoot colour was normal. With low rates of iron the characteristic signs of vanadium poisoning appeared in both crops, and in soybean a horizontal position and an absence of speckling on the leaves was noticeable.

High manganese and molybdenum. Toxic symptoms of both manganese and molybdenum could be detected in this series particularly where the iron supply was low. Apical chlorosis and lower leaf speckling developed in both crops and a golden yellowing in shoot and root of flax, manganese effects predominating on the whole. Increased iron reduced the symptoms, but less so than where the elements were given singly, for soybean became chlorotic with even 20 p.p.m. Fe, and leaf curling was only slight. Dry-weight figures suggest that the effects of the two elements with respect to iron are additive.

High manganese + vanadium. Symptoms of excess of each element developed and no visual offsetting of the manganese symptoms occurred as previously recorded (Warington, 1951). This may have been due to the difference in manganese-vanadium ratio and/or the form and rate of iron provided in the two sets of investigations. Vanadium toxicity symptoms (deepening of shoot colour and root stunting) predominated at the lower iron levels, but manganese effects (lower leaf speckling and upper leaf curling) showed with high iron. Iron presumably counteracted excess of vanadium more readily than that of manganese (at the rates given). Reduction of the former symptoms would then permit the appearance of the latter. On chlorosis, the effect of the two elements was additive in soybean, and this was borne out by the dry weight. A reduction in excess iron symptoms was the supporting evidence with flax. Roots were rather more severely damaged than when vanadium or manganese were supplied alone, the dry-weight figures being somewhat misleading here, as the abnormal thickening apparently increased the yield.

High molybdenum + vanadium. Vanadium toxicity symptoms predominated in this set and the mitigating effects of iron were as previously stated. Roots at low levels of iron were stunted and flax roots were grey-brown.

High manganese + molybdenum + vanadium. This was the most harmful of all treatments. Even the largest amounts of iron failed to prevent apical chlorosis in soybean or allow of normal root development, but it eliminated the early phase of deepened colour (vanadium) and upper leaf curling (manganese) and much reduced the golden yellowing in flax (molybdenum).

The scorings of shoot colour were made only on the youngest fully expanded leaf, but the extent of the chlorosis, as shown by the proportion of yellow and green leaves in soybeans after 5 weeks' growth, closely paralleled its intensity, additive effects again being noticeable (Table 9). Vanadium strikingly influenced the retention of green colour at the base of the shoot where the iron supply was low.

Summarizing the above results; high manganese and molybdenum each caused a further reduction in yield when given in the presence of the other, though the decreases were smaller both absolutely and proportionally than when the elements were given singly. That is, there was a positive interaction between them, most noticeable at the lower iron levels, when the analysis of variance was based on logarithms. A similar interaction was found between manganese and vanadium, but not between vanadium and molybdenum. Characteristic toxicity symptoms of each

TABLE 8. *Dry weights of plants grown with various combinations of manganese, molybdenum and vanadium at different levels of iron.* 1952. (*Mean of 5 plants*)

Treatment	Soybean									Flax								
	2·5 p.p.m. Fe			5 p.p.m. Fe			20 p.p.m. Fe			5 p.p.m. Fe			12 p.p.m. Fe			30 p.p.m. Fe		
(for rates see text)	Shoot (g.)	Root (g.)	Total (g.)	Shoot (g.)	Root (g.)	Total (g.)	Shoot (g.)	Root (g.)	Total (g.)	Shoot (g.)	Root (g.)	Total (g.)	Shoot (g.)	Root (g.)	Total (g.)	Shoot (g.)	Root (g.)	Total (g.)
Control (Mn, Mo, V low)	5·60	1·32	6·92	5·85	1·40	7·25	4·85	1·18	6·03	1·58	0·38	1·96	1·68	0·35	2·03	1·48	0·41	1·89
High Mn (Mo, V low)	0·82	0·25	1·07	0·95	0·27	1·22	2·44	0·68	3·12	0·44	0·15	0·59	0·88	0·26	1·14	1·10	0·32	1·42
High Mo (Mn, V low)	1·02	0·39	1·41	1·40	0·48	1·88	3·51	1·04	4·55	1·07	0·22	1·29	1·32	0·24	1·56	0·83	0·20	1·03
High V (Mn, Mo low)	1·23	0·50	1·73	1·89	0·62	2·51	4·82	1·04	5·86	0·22	0·07	0·29	0·30	0·10	0·41	0·68	0·21	0·89
High Mn+high Mo (V low)	0·67	0·26	0·93	0·73	0·29	1·02	1·09	0·33	1·42	0·42	0·13	0·55	0·85	0·18	1·03	0·89	0·21	1·10
High Mn+high V (Mo low)	0·62	0·28	0·90	0·74	0·33	1·07	1·01	0·31	1·32	0·19	0·08	0·27	0·25	0·11	0·36	0·99	0·21	1·20
High Mo+high V (Mn low)	0·34	0·18	0·52	0·45	0·18	0·63	2·13	0·73	2·86	0·04	0·02	0·06	0·15	0·06	0·21	0·75	0·15	0·90
High Mn+high Mo+high V	0·36	0·16	0·51	0·42	0·19	0·61	0·89	0·38	1·27	0·05	0·03	0·08	0·12	0·06	0·18	0·82	0·18	1·00

TABLE 9. *Effect of excess manganese, molybdenum and vanadium on extent of chlorosis in shoot. Soybean (mean of 5 plants)*

Treatment*	Fe (p.p.m.)	Leaves per plant	
		Total	Chlorotic
Control (Mn, Mo, V all low)	2·5	7·0	Nil
	5·0	7·0	Nil
	20·0	7·0	Nil
High Mn (Mo and V low)	2·5	4·6	3·0
	5·0	5·0	3·0
	20·0	6·2	Nil
High Mo (Mn and V low)	2·5	5·6	4·0
	5·0	5·6	4·0
	20·0	6·6	Nil
High V (Mn and Mo low)	2·5	5·6	3·0
	5·0	6·2	2·0
	20·0	7·0	Nil
High Mn+Mo (V low)	2·5	4·8	3·5
	5·0	5·2	3·5
	20·0	5·4	4·0
High Mn+V (Mo low)	2·5	4·6	3·0
	5·0	5·0	3·0
	20·0	6·0	3·0
High Mo+V (Mn low)	2·5	4·0	2·0
	5·0	4·4	2·5
	20·0	6·4	Nil†
High Mn+Mo+V	2·5	4·0	3·0
	5·0	4·2	3·0
	20·0	5·4	3·0

* For concentrations see § C (1).
† Chlorosis developed 7 days later.

element occurred together and were most pronounced when the iron supply was low. Curling of the upper leaves with excess manganese was an exception, as this seemed to be associated with an adequate iron supply and was, moreover, reduced by the addition of high molybdenum, vanadium or both. This suggested that the curling symptom, like chlorosis, was connected with an abnormal iron-manganese balance and that its prevention by the addition of molybdenum or vanadium might be due to these elements also interacting with the available iron, and bringing the iron-manganese ratio to a more normal level. The effects of all three metals appeared to be additive in respect to iron.

(2) *Incidental effects of micronutrient treatment*

(*a*) *Photosensitivity.* As noted by Merkenschlager (1936), the first pair of leaflets of soybeans were photosensitive, folding up vertically when the light was exceptionally bright. However, in the plants receiving high manganese and low iron (5 or 2·5 p.p.m. Fe) or both high manganese and molybdenum with 2·5 p.p.m. Fe these leaves remained horizontal. Even in ordinary light the upper leaflets of soybeans receiving high vanadium and to a less extent high molybdenum, were held in a more horizontal position than normal. Hopkins, Pagán & Ramírez Silva (1944) also found that the iron supply influenced the photosensitivity of *Phaseolus* grown with excess manganese.

(*b*) pH *changes in nutrient solution.* Though all nutrient solutions were initially at pH 4·6 the reaction changed rapidly on contact with the roots. In cultures where manganese, molybdenum or vanadium were toxic, the pH changes were small, but with the addition of liberal supplies of iron, which counteracted the toxic symptoms, including chlorosis, the trend towards alkalinity was as rapid as in the controls (Table 10). In the latter the iron supply was of little importance. Chlorosis was thus associated with the more acid solutions which would be expected to provide more rather than less available iron.

(3) *Influence of treatment on the iron, phosphorus, manganese, molybdenum and vanadium contents of the shoot*

Analytical data for vanadium, iron and phosphorus were obtained for the shoots of all three crops in selected experiments, but for manganese and molybdenum content the figures were obtained for soybean only. The material was ground in a phosphor bronze mill. Phosphorus and iron were determined colorimetrically, the former by stannous chloride reduction and the latter by the sodium salicylate method. Manganese, molybdenum and vanadium were estimated spectrographically.

Iron. The iron content of the shoots was very low, and often impossible to estimate satisfactorily. However, in soybean, the percentage of total iron in the dry matter was independent of the level of iron supplied where manganese, molybdenum and vanadium were given at the low rate, but was reduced by the addition of any of these elements in excess. The reduction was of the same order in the three cases and was little affected by the concentration of iron provided within any one metal treatment (Table 7). On the other hand, neither high vanadium nor the level of the iron supply had any influence on the iron content of peas, while high vanadium (in the presence of adequate iron) tended to increase it in flax (Table 6). Berger & Gerloff (1947) and Sideris (1950), working with potato and pineapple respectively, found that the iron content of the shoot fell as the supply of manganese was increased, and Nicholas (1952) has described a similar effect with various metals on fresh-water extracts of tomato leaves, though here the iron content of the ash was unaffected.

Manganese, molybdenum and vanadium. The manganese and vanadium contents of the shoot of soybean were greatly reduced by increasing iron in the nutrient solution, as found by Twyman (1951) and Morris & Pierre (1947) for manganese. The effect was most marked when either of the elements was present in excess. On the other hand, no reduction in molybdenum content occurred, except in the

TABLE 10. *Changes in* pH *value of nutrient solution after successive intervals of growth. All initially* 4·6.

Treatment		Peas, 19 May–8 July 1952 (4 plants per bottle)			
		15 days 400 ml.	14 days 600 ml.	14 days 600 ml.	7 days 600 ml.
Low V* (0·1 p.p.m.)	1·25 p.p.m. Fe	5·8	6·7	6·8	6·9
	5·00 p.p.m. Fe	5·9	6·9	6·7	7·0
	20·00 p.p.m. Fe	6·3	6·9	6·9	7·0
High V† (2·5 p.p.m.)	1·25 p.p.m. Fe	4·8	5·5	6·4	6·5
	5·00 p.p.m. Fe	5·2	6·3	6·8	6·8
	20·00 p.p.m. Fe	6·4	7·0	6·8	7·0

Treatment		Soybean 8 July–8 September 1952 (1 plant per bottle)			
		3 weeks 400 ml.	2 weeks 600 ml.	2 weeks 600 ml.	2 weeks 600 ml.
Control‡	2·5 p.p.m. Fe	5·9	6·9	7·1	6·8
	5·0 p.p.m. Fe	5·9	6·9	7·1	6·7
	20·0 p.p.m. Fe	6·8	7·1	7·2	7·1
High Mn‡ (10 p.p.m.)	2·5 p.p.m. Fe	5·7	5·9	6·1	5·7
	5·0 p.p.m. Fe	5·7	5·9	6·2	5·9
	20·0 p.p.m. Fe	6·5	6·8	6·9	6·8
High Mo‡ (40 p.p.m.)	2·5 p.p.m. Fe	5·3	5·5	5·6	4·9
	5·0 p.p.m. Fe	5·5	5·7	6·1	5·3
	20·0 p.p.m. Fe	6·5	6·9	7·1	7·2

* Mean of 10 bottles. † Mean of 20 bottles. ‡ Mean of 5 bottles.

presence of high manganese, in spite of the fact that the additional iron had largely overcome the visual toxic symptoms. Williams & Moore (1952), however, found that the uptake of molybdenum by oats was lower on soils rich in HCl-soluble iron.

The interactions between manganese, molybdenum and vanadium were most marked where the iron supply was low. High manganese caused a rise in both molybdenum and vanadium contents, while high molybdenum or vanadium slightly increased that of manganese (Table 7). In peas and flax, the vanadium content was much reduced by an increase in the iron supply, except in flax grown with only 0·1 p.p.m. vanadium. Peas were evidently more tolerant to vanadium than soybean, for in spite of their high vanadium content they were not seriously damaged by treatment with 2·5 p.p.m. V in the presence of as little as 1·25 p.p.m. Fe (Table 6).

Phosphorus. The effects of high vanadium on phosphorus content were inconsistent and varied with the crop as well as with the iron and phosphate supply. However, where the iron supply was rather low (and the influence of vanadium in consequence accentuated) and the phosphate supply was up to standard level, high vanadium reduced the phosphorus content of shoots of soybean and peas quite considerably in 1951 (Tables 4 and 5) though not in 1952 (Table 6). Flax shoots, on the other hand, showed increased phosphorus content following high vanadium treatment on three occasions (Tables 3, 4 and 6).

IV. DISCUSSION

Johnson (1919) proved that chlorosis in pineapple induced by excess manganese could be cured by spraying with iron. Aiyar (1946), Millikan (1947, 1949), and Morris & Pierre (1947), working with rice, flax and *Lespedeza* respectively, have shown the same effect by addition of iron to nutrient solutions. Willis & Piland (1936) and Shkolnik & Makarova (1950) overcame copper toxicity by iron, while Millikan (1949) showed that zinc, cobalt, nickel but not molybdenum excess could be similarly counteracted. By painting tests, Hewitt (1949) extended the proof of iron deficiency as the cause of chlorosis to yet other metals. The present experiments show that damage from molybdenum and vanadium as well as manganese can be offset by the addition of iron to the nutrient medium. Hewitt (1949) considered that hypotheses concerning interference with iron nutrition based on the relative oxidation-reduction potentials of simple ions were inadequate. Millikan (1950) found that manganese toxicity was increased in solutions containing nitrogen as nitrate or urea, reduced by ammonium nitrate and obviated by nitrogen given as ammonia. This trend was reversed with molybdenum, though some toxicity occurred here with all forms of nitrogen. He suggested that the effect of a heavy metal is related to nitrate and urea but not to ammonia nutrition, the nitrate oxidizing ferrous iron into the physiologically unavailable ferric form. Antagonism between manganese and molybdenum could thus be explained by molybdenum catalysing the reduction of the excess nitrate which accumulates in the presence of high manganese, ammonium ions appearing to facilitate the operation. Hewitt (1951), on the other hand, found that molybdenum accentuated manganese-induced chlorosis whether nitrogen was supplied as nitrate, urea or even ammonia.

An association between iron chlorosis and potassium nutrition is suggested by Jones & Hewitt (1950) who showed that an increase in potash supply diminished chlorotic symptoms in potato, while Wallace & Bear (1949) found a potassium-iron relationship in the sap of alfalfa.

Apart from chlorosis, the effects caused by the different metals in the present investigations were often unlike. Whereas high manganese had little influence on the type of root system (see also Morris & Pierre, 1949), molybdenum induced slight, and vanadium severe root abnormalities. High concentrations of zinc, nickel, cobalt and, particularly, copper can also cause stunting of the roots (Brenchley, 1938; Millikan, 1949; Nicholas, 1952). Since root as well as shoot symptoms of vanadium excess were curable by increasing the iron supply, as Shkolnik & Makarova (1950) found for copper, it would seem that factors besides chlorophyll formation are involved in this iron effect.

The method whereby the toxic effects of manganese and vanadium are overcome by iron appears at first sight to be a reduction in the amount of the metal absorbed by the plant (Table 7), though this fails to explain recovery from chlorosis following external application by iron paints and sprays. Sideris (1950), working with pineapple, found no evidence of precipitation of iron by manganese in the nutrient solution. He attributed the chlorosis induced by manganese to interference with the translocation of iron from the root, as indicated by Epstein & Stout (1951) with tomato. Earlier work (Sideris, Young & Krauss, 1943) had shown that the addition of manganese to the culture solution favoured the accumulation of iron in pineapple roots. The conception of the root as the principal site of the interaction between other metals and iron might thus explain the changes in manganese, vanadium and iron contents of the shoot in the present experiments, though grounds for the inclusion of molybdenum in this hypothesis do not for the moment seem equally clear.

That so many factors influence the results with these chlorosis-inducing metals adds to the complexity of the problem. Both the form in which the element is supplied and the nature of the nitrogen source can alter the toxicity of molybdenum (Millikan, 1950), as also of chromium (Hewitt, 1951). Löhnis (1951) has shown that the damage caused by manganese can be influenced by temperature and the composition of the nutrient solution employed. Apparent inconsistencies between the results from different experiments or of different workers may thus be due to some difference in experimental conditions, particularly as regards iron or nitrogen supply. In view of this, special caution would seem to be required in the use of such terms as 'threshold of toxicity' without sufficient qualification.

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EXPLANATION OF PLATES 1 AND 2

(Photographs by V. Stansfield)

PLATE 1

Fig. 1. Pea. Standard P and high (20 p.p.m.) Fe. (L–R) 0·1, 2·5, 5 p.p.m. V. Fe overcomes V toxicity.

Fig. 2. Standard P and standard (5 p.p.m.) Fe. (L–R) 0·1. 2·5, 5 p.p.m. V. Fe too low to overcome V toxicity.

Fig. 3. Pea. Standard P and low (2·5 p.p.m.) Fe. (L–R) 0·1, 2·5, 5 p.p.m. V. Fe too low to overcome V toxicity.

Fig. 4. Pea. $\frac{1}{10}$ P and high (20 p.p.m.) Fe. (L–R) 0·1, 2·5, 5 p.p.m. V. Fe less effective in overcoming V excess than where standard P supplied.

Fig. 5. Pea. Low (0·1 p.p.m.) V. (L–R) 1·25, 5, 20 p.p.m. Fe. Rate of Fe of little importance.

Fig. 6. Pea. High (2·5 p.p.m.) V. (L–R) 1·25, 5, 20 p.p.m. Fe. Increase of Fe overcomes V toxicity.

Fig. 7. Flax. L group. Low (0·1 p.p.m.) V. R group. High (2·5 p.p.m.) V. (L–R) 5, 12, 30 p.p.m. Fe. High Fe overcomes V toxicity, but rate of Fe of no importance where V low.

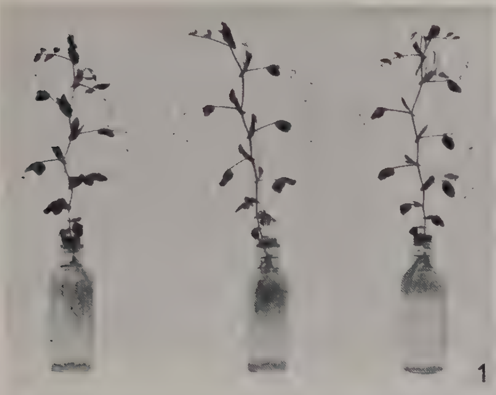
PLATE 2

Fig. 8. Soybean. Control. Low Mn, Mo and V. (L–R) 2·5, 5·0, 20 p.p.m. Fe. Rate of Fe of no importance.

Fig. 9. Soybean. High Mn. Low Mo and V. (L–R) 2·5, 5·0, 20 p.p.m. Fe. High Fe removes Mn-induced chlorosis, but may increase leaf curling.

Fig. 10. Soybean. High Mo. Low Mn and V. (L–R) 2·5, 5·0, 20 p.p.m. Fe. High Fe removes Mo-induced chlorosis.

Fig. 11. Soybean. High V. Low Mn and Mo. (L–R) 2·5, 5·0, 20 p.p.m. Fe. High Fe removes V-induced chlorosis and improves root growth.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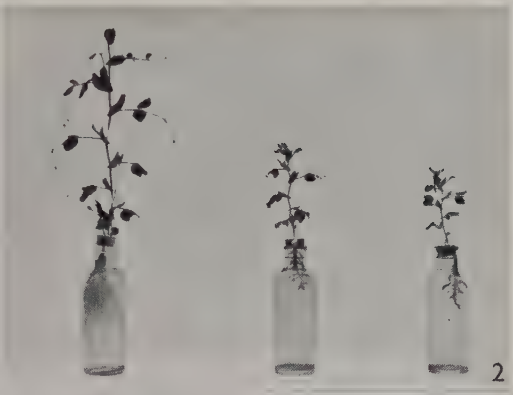
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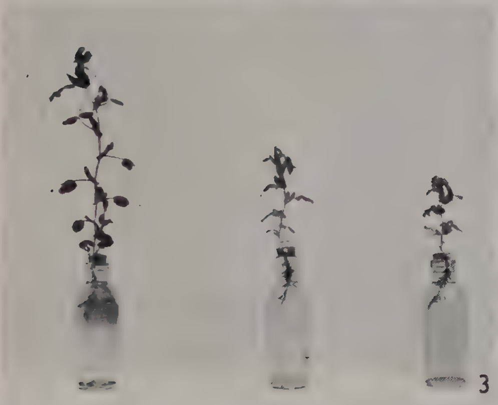
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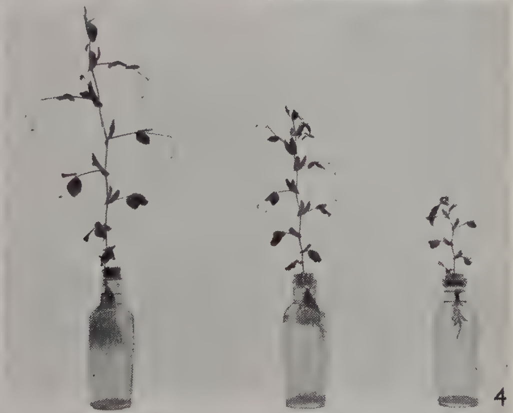
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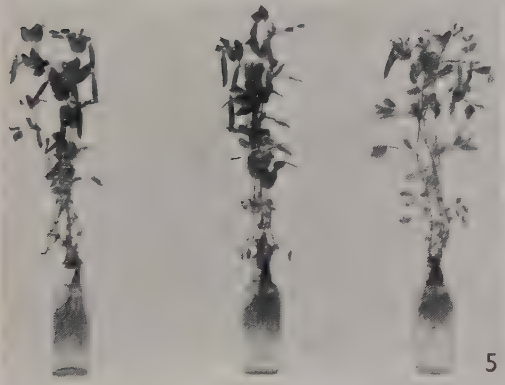
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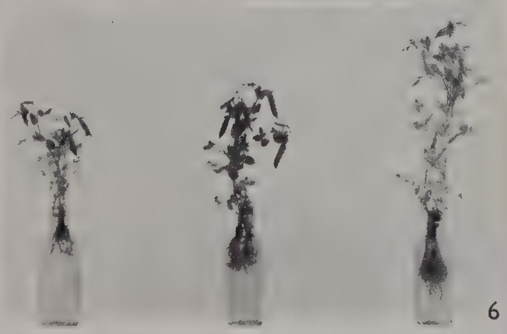
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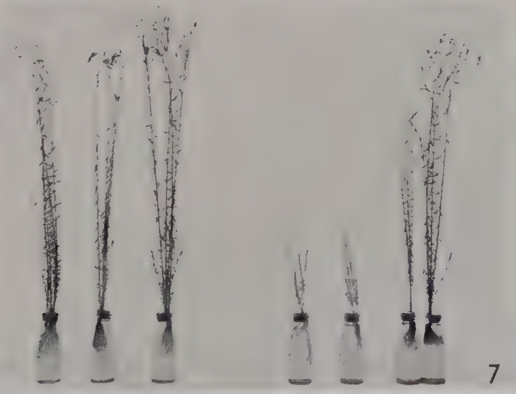
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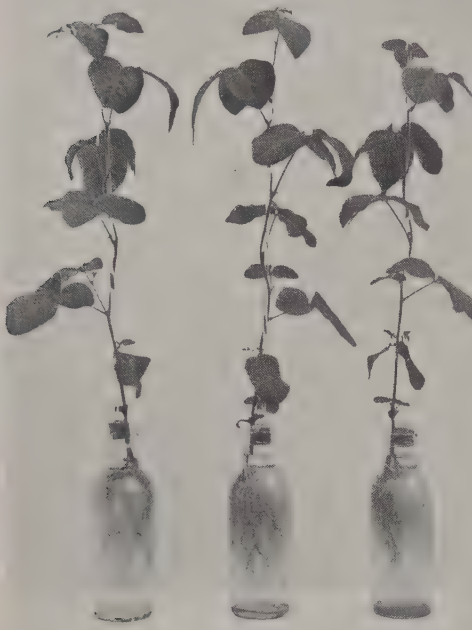
WARINGTON—*Influence of iron supply*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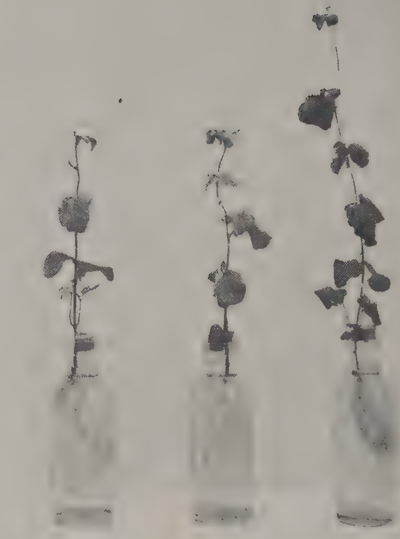
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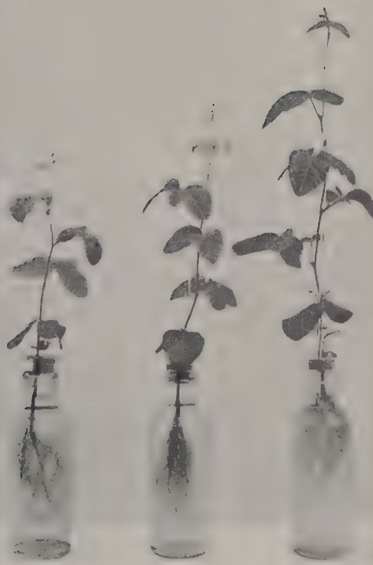
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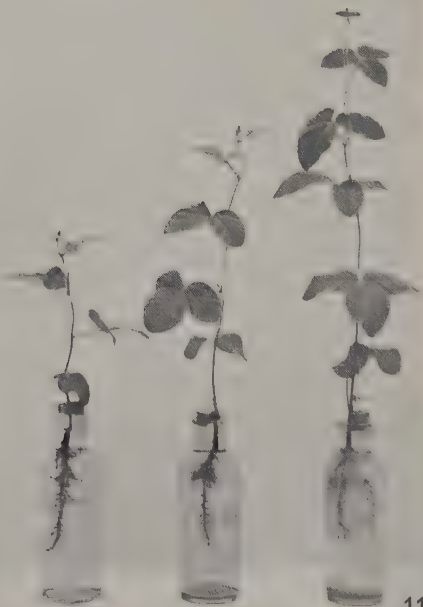
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WARINGTON—*Influence of iron supply*

Nutman, F. J. & Roberts, F. M. (1954). *Ann. appl. Biol.* **41** (1), 23–44.

VALSA EUGENIAE IN RELATION TO THE SUDDEN-DEATH DISEASE OF THE CLOVE TREE (*EUGENIA AROMATICA*)

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Clove Research Scheme, Zanzibar

(With Plate 3 (in colour), Plate 4 and 6 Text-figures)

The sudden-death disease of the clove tree is invariably associated with the fungus *Valsa eugeniae*. The pathogenicity of this fungus has been investigated experimentally, and it has been shown that it is a primary parasite on mature clove trees, that saplings are resistant to it, and that seedlings are immune. It has been experimentally demonstrated that water-borne spores of the fungus can invade the absorbing and the fibrous roots of the clove tree. The slow-decline disease, which affects clove saplings only, is associated with a slow and progressive root-rot over a period of many years. *Valsa* is also invariably associated with this disease, which occurs only in areas which have been replanted after the previous stand has been killed by sudden death. It is thought that slow decline is the symptom-expression of *Valsa* attack on young trees when these still retain some measure of juvenile resistance to it.

The history of the sudden-death disease of clove trees in the Zanzibar Protectorate, and of the investigations into its cause over the last 30 years, has been fully reviewed by Nutman & Roberts (1953*b*). One of the more recent studies of the problem was by Campbell (1940), who dismissed the possibility that sudden death might be caused by a fungus, or any other pathogen, and asserted that it was physiological. Nutman & Sheffield (1949), however, studied the epidemiology of the disease, and concluded that it was caused by a pathogen, possibly a virus. Much work was done on the virus hypothesis from 1948 onwards by these workers and others until, towards the end of 1950, convincing evidence was obtained that a fungus was associated with the disease. At about this time, also, strong evidence against the virus hypothesis became available as a result of the grafting work of May (1949). Many grafts of diseased scions to healthy stocks had been made, and all had failed, after being established for up to 3 years, to transmit the disease. In addition, no positive evidence was obtained from any type of transmission experiments, or from physiological and histological studies.

FIELD OBSERVATIONS

The symptoms of sudden death have been fully described by Nutman & Sheffield (1949). The classic symptoms are a heavy green-leaf fall, followed by the sudden wilt of the remaining leaves, and then by their drying to a russet colour while still on the tree (Pl. 3*a*): these are usually seen at their best in groves of mature (over 30-year-old) trees with unbroken canopy. Symptoms vary, however, possibly depending on moisture conditions preceding death. Infection can sometimes be detected, for example, by a yellowing and thinning of foliage before the onset of the final symptoms.

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* Seconded from Rothamsted Experimental Station.

The one unvarying symptom in mature trees is that the finer roots, including the absorbing roots, have degenerated by the time the trees show the first slight indication of disease. It is not known for how long this precedes death, but there is evidence that trees may be affected for many months before any external signs can be recognized. After death, and after the russet leaves have been beaten off by wind and rain, the trees, too often left standing to provide shade for young replants, have a silver-white appearance, and these, sometimes covering many acres, are now a conspicuous feature of the Protectorate landscape.

Standing clove trees show no external sign of fungal infection until 3–4 months after death. The wood of the clove is exceptionally hard and resists decay, often for many years; even then the cause of final disintegration is often termite attack. Internal evidence of the presence of a fungus, however, can be seen a few days to 2 weeks after death by slashing the collar, when a bright yellow stain is visible in the cambial region. This stain later spreads rapidly up the trunk in a progressively broadening band, until, in a tree which has been dead for about 9 months it can be found distributed generally throughout the trunk and branches (Pl. 3*b*). Immediately after death, however, the yellow cambial stain can be found in individual main lateral roots at varying distances from the collar; in trees which are almost dead, it is sometimes found only at considerable distances from the collar, in one root, for example, at 22 ft. A proportion of the smaller roots, however, are decayed and often intensely yellow, fibrous roots have often lost their outer cortex, and absorbing roots are absent. Preceding the appearance of the yellow stain, a greyish discoloration of the cambium can sometimes be observed some distance in advance of it. This discoloration is not always clear and should not be confused with the darkening of the cambium which always takes place when a tree is slashed. When a symptom of infection it can be seen at the moment of exposure, whereas in healthy trees it takes several minutes to develop.

This yellow stain, always associated with clove trees which have died of sudden death, does not occur in the wood of those which have died from other causes, such as infection with *Cryptosporella eugeniae*, the main cause of dieback (Nutman & Roberts, 1952), from waterlogging, drought or fire damage.

Approximately 3–4 months after the death of a tree, small black perithecia begin to develop, at first at ground-level, and later, following the yellow stain, over all the upper branches. These fructifications are not, at first, conspicuous; they occur, however, in enormous numbers, causing the bark to crack, often in a somewhat rectangular pattern, with the perithecial necks, sometimes half-a-centimetre in length, protruding in lines or tufts. They have never been found below ground-level, although they will develop freely on exposed roots either in the field or in moist chambers in the laboratory.

Pycnidia rarely, if ever, occur on undamaged parts of a dead tree but, in damp conditions, they develop in enormous numbers on the cut ends of stumps or on the surface of any cut which exposes the wood. When a tree has been felled very soon after death they are at first confined to a ring in the region of the cambium. When felling is delayed, however, they develop over the whole cut surface of the stump. In fact, like the perithecia, their distribution coincides with that of the yellow stain.

These fructifications are of *Valsa eugeniae* Nutman & Roberts, and they have been fully described elsewhere (1953). The water-soluble yellow stain is a product of this fungus, and it is also produced in pure culture on potato-dextrose agar.

A detailed survey was made in January-March 1950 of many sudden-death areas in the islands of Zanzibar and Pemba. Six hundred dead trees were examined, and all but two of these contained *Valsa*. The exceptions, both from an area where dieback is prevalent, had been girdled by *Cryptosporella eugeniae* which has been shown by Nutman & Roberts (1952) to be capable of causing death in the sudden-death manner when the attack is in the main trunk. Since this survey, many hundreds more sudden-death trees have been investigated, and in every one of these *Valsa* has been found.

Many isolations from the roots and trunks of sudden-death trees have been made, and *Valsa* has always been obtained without difficulty in pure culture. In addition, moist chamber cultures of infected wood almost invariably yield this fungus only. It is usually the only one present in the wood of trees which have died from sudden death, and pure cultures have often been obtained, without transfer, from plating lightly flamed fragments of infected wood in potato-dextrose agar.

A detailed survey was made of a group of eighty dead trees whose dates of death had been recorded over a period of 3½ years. In every sudden-death tree the characteristic yellow stain was found, and in each instance its development was from the roots upwards, reaching the collar as a band near the cambium a short time after death. One tree, not yet quite dead, showed traces of yellow at the collar. Seventy-three bore perithecia of *Valsa*, and these had all been dead for a minimum of 3 months. Functional perithecia were found at all levels on trees which had died 29 months before. Two trees in this block had been killed by *Cryptosporella*, and these had no yellow stain or perithecia.

Groups of dead trees, killed by causes other than sudden death, were examined in three widely separated districts in Zanzibar island. The first was on an isolated ridge towards the north, which is still relatively free from the disease. The trees had been killed by fire some 2 years previously. Some scores were examined, but no trace of *Valsa* could be found. The second, also to the north, had again been killed by fire and, although near to a small but active outbreak of sudden death, was also found to be free from *Valsa*. In the third district, which was of considerable size and in which a small amount of sudden death was already present, inundation followed by drought killed all the trees in 1946. In 1951, although some of the trees might have been infected before the inundation, and although the stumps had been exposed to casual infection for 5 years, it was found that *Valsa* was present in 40% of them only, 60% being free from it.

DETAILED STUDY OF DISEASED TREES

A study was made of eight clove trees, ranging from two which showed only slight symptoms to others which had just died or were near death. Two trees, one of which had been dead for 2 weeks, and another for between 1 and 2 months, were included. Healthy trees were also examined. All were at least 30 years old, and some were considerably older.

The trees were felled, and the boles with 3–4 ft. of the main lateral roots were dug up and washed. In some instances, particularly when dealing with trees in the early stages of the disease, two or more main roots were excavated for considerable distances. The boles were sawn across at the collar, and also at varying distances from it, depending on the position of the main roots. The latter were also sawn across at varying distances from the trunk and the cut surfaces planed and sanded to show the distribution of stain. Diagrams of some of the sections of one of these trees are shown in Text-fig. 1. In the figure, the uneven distribution of the yellow stain in the main laterals and collar shows clearly, as does its general peripheral distribution. Of particular interest are the two diagrams of root no. 7, which was a small secondary from a main lateral, showing the infection of the larger root from the smaller. The distribution of hyphae in some of these sections is also shown.

Small sample blocks were cut from the main sections of the collars, main roots and small secondary roots at various points. After being slightly softened by boiling they were sectioned, stained in safranin and boiling picro-aniline-blue, and mounted in euparal. This stain, when used correctly, gives brilliant blue hyphae in contrast with the red xylem elements and reveals the presence of hyphae in sections where cotton blue in lactophenol will show none.

The following is a summary of the results obtained:

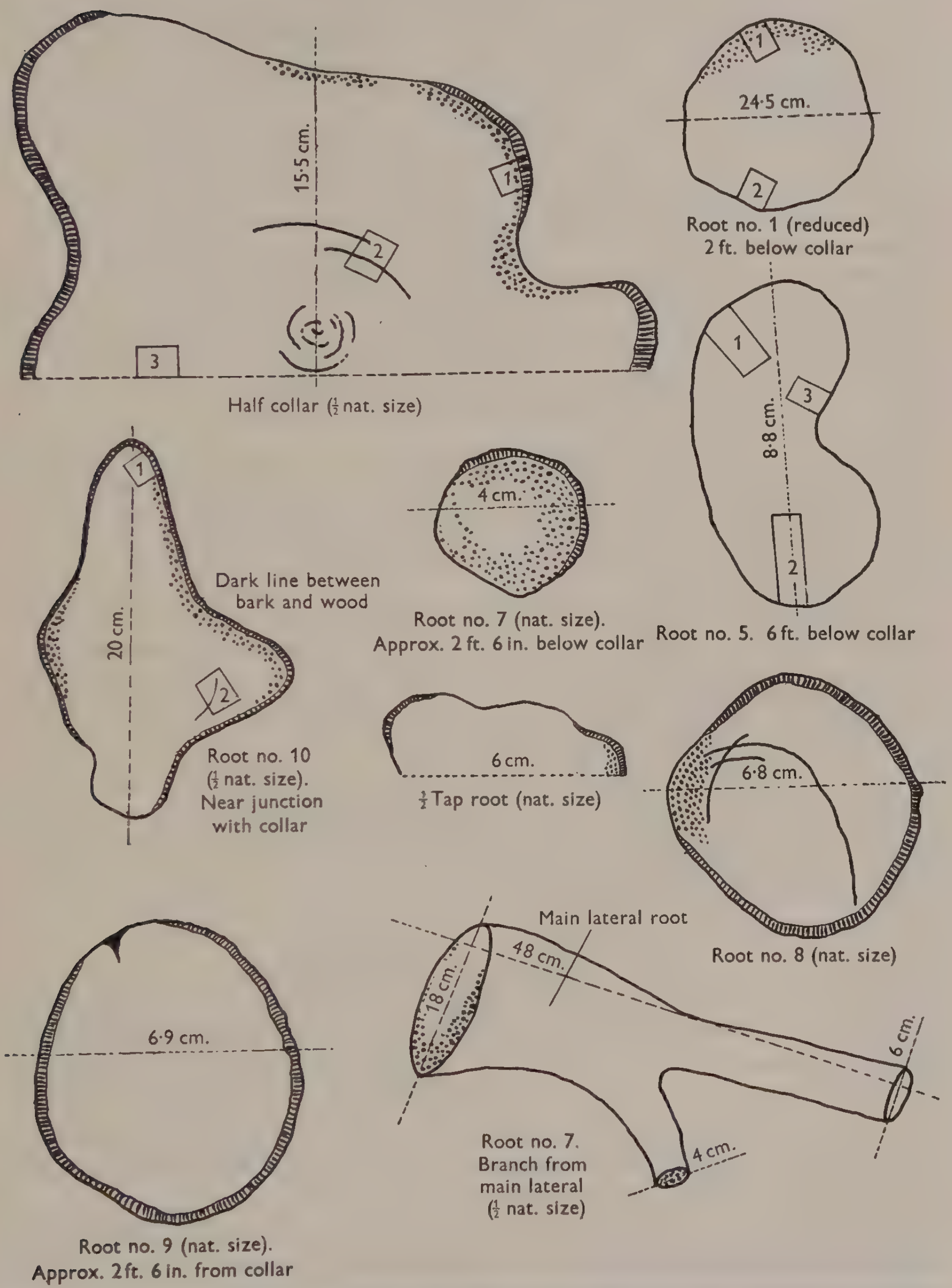
Group 1. Trees not yet dead, ranging from those whose leaves are as yet unwilted, to those where the final wilt has just begun.

No. 1. No yellow stain in any part of the main roots examined. Hyphae found in one main lateral 14 ft. from collar, but very sparse, and only near cambium. At 22 ft. from collar, hyphae more numerous, in both phloem and xylem, but only near cambium. In small secondary roots, at varying distances from collar, hyphae much more numerous.

No. 2. No yellow stain: no hyphae in the main roots nearer to the collar than 6 ft., and then only in the cambial region. Secondary roots showed more numerous hyphae, in cambial region only.

No. 3. No yellow stain: no hyphae in main roots nearer to collar than 6 ft. and then only near cambium. In one secondary root, 4 ft. from collar, hyphae very numerous but only near cambium.

No. 4. No yellow stain: no hyphae in main laterals nearer to collar than 2 ft., and then only in cambial region. As in the other trees, the secondaries contained more hyphae.


15·5 cm.
1
2
3
Half collar (½ nat. size)
1
24·5 cm.
2
Root no. 1 (reduced)
2 ft. below collar
1
3
8·8 cm.
2
Root no. 5. 6 ft. below collar
1
20 cm.
2
Dark line between
bark and wood
Root no. 10
(½ nat. size).
Near junction
with collar
4 cm.
Root no. 7 (nat. size).
Approx. 2 ft. 6 in. below collar
6 cm.
½ Tap root (nat. size)
6·8 cm.
Root no. 8 (nat. size)
6·9 cm.
Root no. 9 (nat. size).
Approx. 2 ft. 6 in. from collar
Main lateral root
48 cm.
18 cm.
6 cm.
4 cm.
Root no. 7.
Branch from
main lateral
(½ nat. size)

Text-fig. 1. Selection from roots of tree no. 5. Stippling indicates yellow stain. No hyphae were found at points 2 and 3 in the collar.

Group 2. Trees which had either just died or were nearly dead.

No. 5. Distal parts of the main roots strongly stained, the stain diminishing towards the collar where it was peripheral and patchy, the patches being associated with main laterals. The distal parts of the roots contained hyphae in all tissues; these decreased in quantity towards the collar and, in the basal ends of the roots, were confined to the cambial region. In the collar, hyphae were confined to the region of the yellow stain. In the roots of this tree the invasion of the main roots, via the secondary ones, was very evident (see Text-fig. 1).

No. 6. Almost exactly as no. 5.

No. 7. Somewhat younger than most of the other trees. Yellow stain in peripheral parts of the root system, reaching the collar as a narrow band near the cambium. At the collar, the cambium was more blackened than normal, due to an unusually heavy deposit of 'gum' in the adjacent cells. Hyphae were present in all tissues of the roots and collar.

No. 8. A tree which had died 14 days previously. Yellow stain conspicuous, strongly staining the more distal parts of the main roots, and extending to 1 ft. above the collar as a narrow peripheral band. Hyphae present in all parts of the main roots, but less numerous in the centre of their basal portions. In the trunk hyphae present in association with the peripheral yellow stain, but absent in the centre.

That the fungus which can be demonstrated in the main lateral roots of trees, such as those of group 1, is the one responsible for the subsequent development of the yellow stain, was shown by placing pieces of the unstained roots in moist chambers where they invariably developed yellow stain, followed by pycnidia and perithecia of *Valsa*.

In healthy trees, or in parts of diseased ones where no mycelium can be found, there are very few tyloses, one such tree having one tylosis only in 154 vessels examined. As the fungus advances up the roots, however, a wave of tylosis-formation precedes it; in one example, where hyphae were present but sparse, 82 vessels out of 175 were completely occluded.

It is rare to find abundant tyloses and abundant hyphae in the same region. Invasion of tyloses by hyphae has been occasionally observed followed by their collapse. The same phenomenon can be seen, even more clearly, in cases of dieback caused by *Valsa*, or in experimental infections.

VALSA EUGENIAE AS A CAUSE OF DIEBACK

Valsa occurs as a wound parasite on clove trees where it causes a dieback rather similar to, but slower in development than that caused by *Cryptosporella eugeniae* (Nutman & Roberts, 1952). It is also less common. For example, in one plantation of 253 trees in a sudden death-free area, 6162 dieback infections were found to be caused by *Cryptosporella* and only 627 by *Valsa*. The significance of the latter in the role of a wound parasite, however, may be of more importance that its relative incidence would indicate, and this will be discussed later.

Unlike *Cryptosporella*, which seems to be able to become established in a clove tree through any wound or even abrasion, *Valsa* rarely enters except through branches which have been broken in such a way as to leave a splintered wound. Such damage is often caused, particularly to upright branches, during harvesting. It is fairly common to find this type of wound infected with both fungi, the respective limits of spread being clearly indicated by the two different coloured stains which they produce.

When an invaded branch is split open, or a trunk infection exposed, the wood nearest to the entry point is seen to be stained yellow, the size of this area depending on the age of the infection. In advance of the yellow area, and, in main trunks often considerably in advance, are greyish or blackish discolorations which generally occur in a fairly narrow zone but sometimes extend for many inches, in the form of isolated flecks. Microscopically it can be seen that belts of xylem elements are occluded by a gummy substance, tyloses are very numerous, and very scanty mycelium can often be found. *Valsa* will readily grow out of such flecked samples when these are placed in moist chambers.

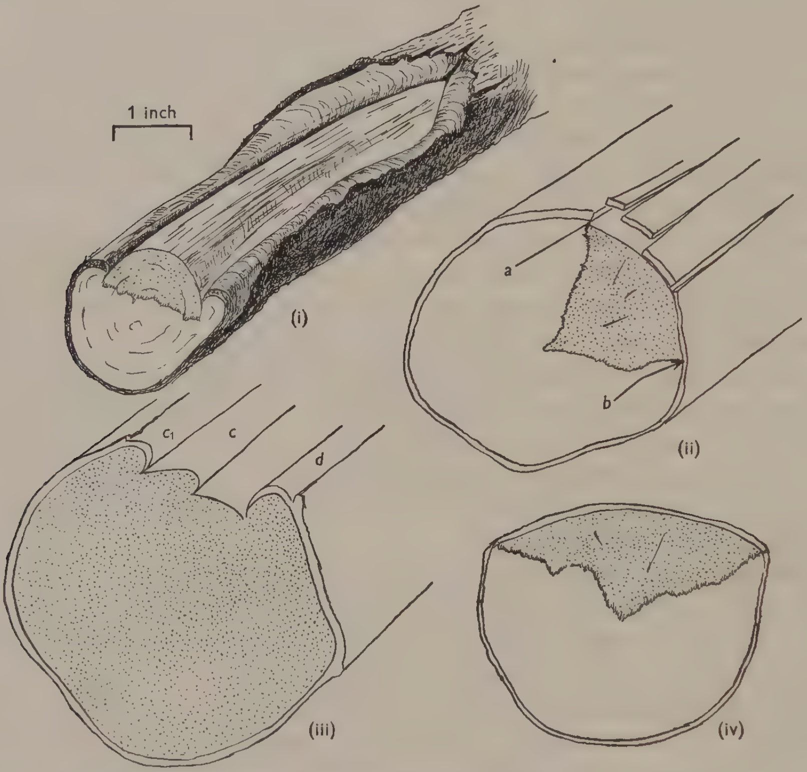
Some measure of resistance to invasion by *Valsa* is shown by a type of lesion on branches or trunks which is locally common. Linear depressions, sometimes many feet in length, can be seen running up the trunks and along the branches, often on their lower sides. These are caused by the fungus advancing up a sector only. Regeneration along the edges of the infected area, and normal growth of the uninfected parts, causes the formation of the depression and finally, when the old bark over the lesion drops off, to long narrow grooves. Subsequently the regenerated wood often becomes invaded; Text-fig. 2 (iii) shows an instance where this has happened twice. One edge of a lesion may show new growth, while on the other the fungus may develop unchecked.

Microscopically, no physical barrier between the infected and the healthy wood can be found. In both active and inactive lesions the mycelium is confined to the stained area, with profuse formation of tyloses just outside its boundary and with the cells of the infected zone heavily charged with gum.

V. eugeniae, and possibly one other unidentified fungus, is also associated with a comparatively rare condition which is best described as 'small twig dieback', severely affected trees being outlined by a halo of white terminal twigs. This is usually seen when the trees are in poor condition, and it is not thought to be of any great importance. Usually, infection originates in the small twigs from which the bud clusters have been picked, but generally the fungus ceases to grow back after reaching a node on a larger twig. Very rarely, growth continues, and dieback of a larger branch is caused.

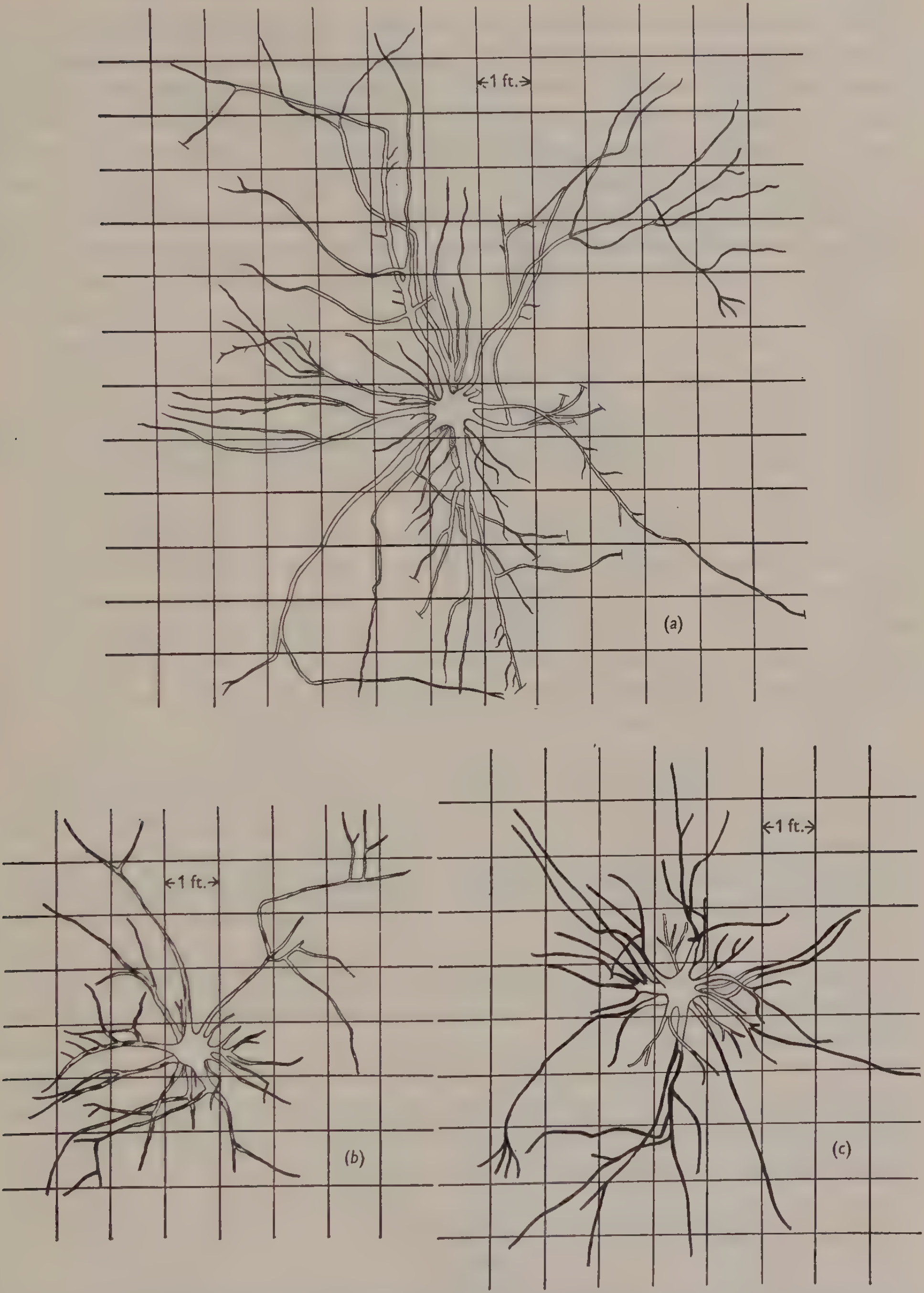
SLOW DECLINE OF YOUNG CLOVE TREES

One of the most disturbing aspects of the clove industry at the present time is the steady increase of a disease which we now describe as slow decline. This occurs in young saplings and becomes noticeable usually at about the age at which they begin to produce their first small crops. The first sign of the disease is a general slight unthriftiness. Later, the leaves become progressively reduced, both in number and size and are lighter in colour than those of healthy trees. Steady deterioration continues, generally for several years, the trees often showing temporary apparent improvement. Death may take place comparatively suddenly, in which case many leaves wilt and dry up while still attached to the tree, but more commonly the process is very gradual and the tree does not die until it is almost completely defoliated. (Pl. 4*a*).



Text-fig. 2. Typical *Valsa* lesions on branches. Stippling indicates yellow stain. (i) Healthy regeneration on both sides of a lesion. No completely healed one has been seen, so presumably they all break down eventually. (ii) Active lesion, showing beginning of probable regeneration at *a*, but more at *b*. (iii) Lesion which had regenerated twice on one edge (at *c* and c_1), and once on the other edge at *d*. The fungus eventually invaded both edges, and subsequently girdled and killed the branch. (iv) Transverse section through typical lesion, with no regeneration, showing distortion produced by cessation of growth in the affected sector.

Campbell (1940) described this condition as 'immature tree dieback' in contrast with dieback of mature trees. In root exposures he observed that the majority of the main lateral roots were dead, and tentatively suggested that the cause might be fungal. Nutman & Sheffield (1949) described the slow decline condition as one of the forms of sudden death, when young trees, up to 20 years of age, were being attacked.



Text-fig. 3. Root systems of three trees in a plantation affected by slow decline (1 ft. grid). Tree (*a*) was apparently healthy; tree (*b*) was showing the first signs of decline; and tree (*c*) was seriously affected. The rotted portions of the roots are black in the diagram.

The disease, so far as our observations go, is to be found only where young trees have been replanted in areas previously devastated by sudden death. It has never been found in plantations of young trees on virgin ground, or in areas where sudden death does not occur. When the root systems of a representative selection of young trees in a severely affected plantation were exposed by hand, the root-rot, as described by Campbell, was present in every one. Text-fig. 3 illustrates the root systems of three comparably aged trees, one very near death, one showing symptoms and one healthy. Troup (1931) observed chafer attack on 'sudden death' roots, and suggested that this might be a cause of the disease, but Kirkpatrick later (1932) showed that these larvae will not feed on the roots of healthy clove seedlings. We have often observed chafer damage on the roots of slow decline trees but never on the roots of true sudden-death casualties, and it seems possible that the trees which Troup examined were affected by slow decline.

When the roots and collars of such trees are slashed immediately after death, a greyish discoloration with a somewhat streaky distribution can be seen near the cambium. This is followed, after an interval, by the typical *Valsa* stain and later by fructifications. Samples of their roots, taken in advance of the rotted portion, and placed in moist chambers, nearly always develop *Valsa* fructifications. These take longer to form on this material than on comparable samples from typical sudden-death trees. This might indicate that the mycelium, growing in young and, as will be shown later, resistant material, is comparatively scanty. Contaminating fungi (as would be expected in roots which have been rotting slowly) are numerous, one in particular, apparently a *Diplodia*, being very frequent.

In a Government plantation of known history this disease was studied in some detail. The trees had been established in an area which had previously been devastated by epidemic sudden death, and as the block was used for a manurial trial, they had been carefully tended. Until a few years ago this plantation of young clove trees was undoubtedly one of the finest in Zanzibar island, but early in 1950 symptoms of slow decline began to appear. In 1952 it was decided to remove every alternate row, and this gave access to ample material. The root systems of every fifth tree, to a total of forty-one, were examined, and typical root-rot was seen in every one. Samples of the roots were placed in moist chambers, and the presence of *Valsa* was confirmed in thirty-five.

No root-rot could be found, and no *Valsa* obtained from extensive sampling of roots of young trees, of comparable age, from two widely separated plantations still free from sudden death. One of these was known to have been planted on virgin ground, and so, very probably, was the other.

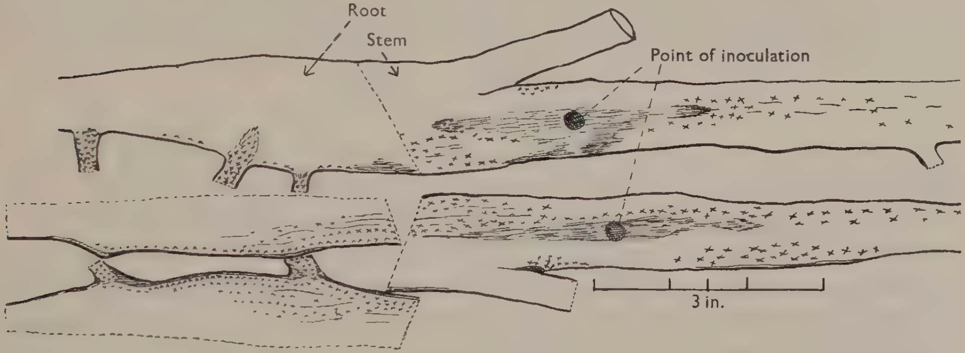
EXPERIMENTS ON THE PATHOGENICITY OF *VALSA EUGENIAE*

Young seedlings

Numerous experiments have been carried out on young seedlings of up to 1 year old. These have been inoculated with pycnospores, ascospores and mycelium, singly and in combination, using several methods, but without success. In one experiment the roots of seedlings, grown in moss, were rubbed with carborundum, sprayed with a spore suspension, and kept in moist conditions. In no instance was any plant visibly affected by the fungus. Negative results were also obtained from experiments using potted seedlings, 4–5 years of age. These were inoculated by various methods, in the collar regions and in the roots, with mycelium from pure cultures. No infections were ever obtained. It seems evident, therefore, that clove plants, up to about 4 years of age, are immune from experimental infection by *Valsa*, and this immunity is confirmed by field evidence.

Young saplings

A ring of $\frac{3}{16}$ in. holes was drilled round the main trunks of each of twenty-four 9-year-old saplings, and these were immediately flooded with a suspension of *Valsa* spores. Relatively large quantities of the liquid were absorbed. An equal number of controls was flooded with water. Soon after inoculation, discoloration indicated that invasion was taking place, and hyphae were found around the holes. These holes, however, eventually healed, and the trees showed no further indications of being affected.



Text-fig. 4. Longitudinal section through a young tree killed by inoculation with *Valsa*. The distribution of yellow stain is shown by stippling, and that of black discoloration by shading.

This experiment was repeated several times, using in some instances rings of closely spaced holes made with a dentist's drill. Similar results were always obtained, with one exception. This was a pot-bound 5- to 6-year-old tree, which had been grown in a large metal container in the laboratory. Inoculum was introduced through a single $\frac{3}{16}$ in. hole in the trunk and, 7 months later, the tree died after shedding most of its leaves, a comparable control tree remaining healthy. When split open, the sapling was seen to have been girdled by *Valsa*, which later was recovered from it (Text-fig. 4).

Branches of young saplings

Three branches on each of six very vigorous 9-year-old saplings were twisted until longitudinal cracks developed in the bark. Three branches on each of six similar trees were bent until the bark on the convex side just fractured. Half the treated branches were inoculated by flooding the cracks with a suspension of *Valsa* spores, and the other half served as controls.

Seven months later, two branches were dead, and *Valsa* was re-isolated from them, and after 8 months, five were dead. Eleven months later, eight branches were dead and thirteen were infected but not yet dead. All controls remained healthy, except three which acquired casual infections with *Cryptosporella.*

This experiment was repeated with fourteen similar saplings, six of which were controls. Preliminary results were satisfactory, but, 18 months after the start of the experiment, the trees were harvested, and of the twenty-four treated branches, thirteen were broken off by the clove-pickers. The eleven remaining branches were all infected, and seven of them dead. In four the infection had either reached the main trunk or was within 1 in. of it. All the remaining controls remained healthy.

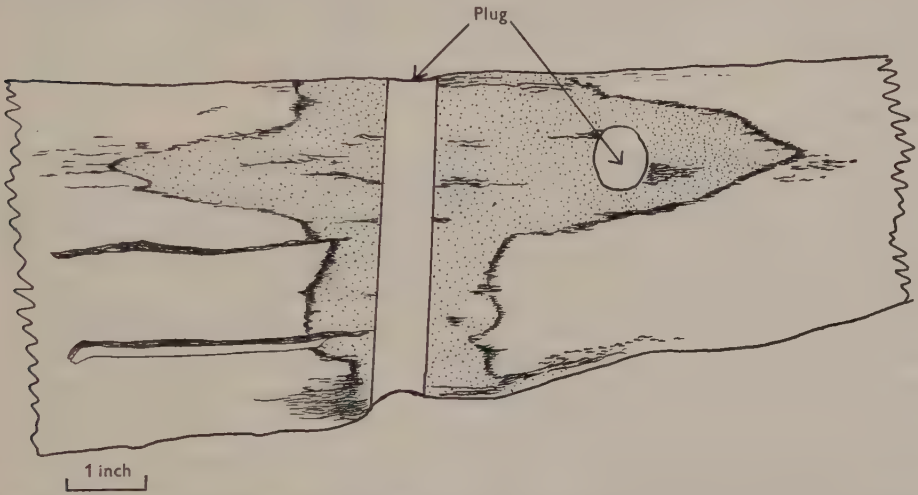
Mature trees

Eugenia aromatica is a very slow-growing and valuable tree, coming into full bearing only after about 20 years. Consequently the number with which it was possible to experiment was limited, particularly since inoculation experiments could not be done on any privately owned plantations. Further, the progress of inoculation experiments, except at considerable intervals, could not be followed, for this would entail the sacrifice, at perhaps too early a stage, of the limited experimental material. Two plantations were available. One of these had the disadvantage of being in a sudden-death area, and the other was in an area free from sudden death but in which *Valsa* occurred as a wound parasite; before experiments started in the latter a pruning campaign greatly reduced the quantity of fungus present, but it was impossible entirely to eliminate it.

Experiment 1

Twelve mature trees, 25–30 years old, were inoculated by drilling a ring of $\frac{3}{16}$ in. holes, about 1 in. deep and $1\frac{1}{2}$ in. apart, between the collars and the first branch or fork. A dense suspension of spores and broken-up mycelium from a pure culture was introduced through a pipette, the holes being filled repeatedly until suction slowed down. Twelve control trees were treated with water. All holes were plugged with corks or plasticine.

One tree was sampled after a month by excising from the trunk a block of wood containing one hole. The area immediately around the hole was darkened, and showed a slight yellow stain near the outskirts. Mycelium occurred in the vessels a few centimetres above and below the hole, and for a few millimetres only on either side. Cultures yielded typical *Valsa* fructifications. Additional samples taken for the next 17 months continued to show slow but steady spread. After 21 months samples 8 in. long, which included several holes were taken from each of four trees, but they did not show the limits of the stained area. Mycelium was present in and near the cambium above and below the holes and was invading newly formed wood near the edges of the holes. Samples taken at the same time from the control trees were slightly discoloured immediately around the holes, but showed no hyphae and did not yield *Valsa*.



Text-fig. 5. Longitudinal section through a root which has had two pegs of *Valsa*-infected wood driven into holes bored in it. The area of yellow stain is stippled.

Experiment 2

A trench, 2 ft. wide and 2 ft. deep was dug by hand, without severing roots, around each of nine mature trees 4 ft. from the trunk. Holes, $\frac{3}{16}$ in. in diameter and $\frac{1}{2}$ in. deep were drilled in all the larger roots, and smaller ones in the smaller roots. The trenches were then filled with leaf mould and, after some months, covered with soil. Six of them were watered with a suspension of *Valsa* spores for 1 month at 2-day intervals, and later less frequently. Three of the trenches had water applications only.

After 14 months one main root taken from each treated tree showed considerable discoloration, not only near the holes but extending, in the form of faint grey flecks, several inches on either side. Hyphae and numerous tyloses were present in the discoloured area and some of the holes had been partially sealed by callus which had sometimes been invaded by the fungus. Cultures from wood at the edge of the discoloured zone gave typical fructifications of *Valsa*.

Experiment 3

Six approximately 50-year-old trees were each wounded by a ring of ½ in. holes bored at a slight downwards slope, just above the collar. These were filled with spore suspensions immediately after drilling and again at short intervals for a month. After 5 weeks samples taken from two of the trees showed slight spread of the fungus from the holes, with a typical black boundary line characteristic of *Valsa*. After 1 year, further sampling showed that development, similar to that in Exp. 1, was continuing.

Experiment 4

A ring of 1 in. squares of bark, about 2 in. apart, was removed from the trunks of mature trees by making cuts with a sharp chisel down to the cambium. After inserting mycelium and spores from a pure culture, the bark was replaced, and firmly bound with strong twine. Three different groups of trees were inoculated at different times. The first two control trees became infected, and it was subsequently found that the source of contamination was several branch snags affected with *Valsa* above some of the points of inoculation. Consequently, two more control trees were prepared; in these the bark was washed with a solution of mercuric chloride before cutting with sterilized tools and, after the bark squares were back in place, a wide 'skirt' of waterproof Cellophane was tied firmly to the trunk above them, and luted to it with lanoline. These precautions were successful, none of the excised squares of tissue ever becoming infected.

In one of the two trees inoculated in June 1951, the wet appearance of the cambium, characteristic of *Valsa* attack, was obvious after a week. Later the usual signs of fungus development could be seen originating from any square examined. After 7 months this tree began to show signs of decline, such as slight yellowing and thinning of foliage, and was apparently almost girdled. A period of apparent recovery followed the rains, but after 18 months the tree is obviously dying, and one branch has already died. Extensive yellowing can be traced for several feet up the trunk. The second tree of this pair, apparently comparable in every way with the first, so far shows no ill effects, and callus formation is proceeding at all the inoculation points.

One tree of the group of four inoculated in August 1951 showed, at one inoculation point, a longitudinal spread of 9–10 in. above and below the point of inoculation after only 21 days (Pl. 4, fig. b_2). After 3 months, two points on two different trees showed spread of 8 and 5 in., while another showed good development with the characteristic black boundary line.

Two points on the third and largest group of trees (ten trunks, treated in October 1951) showed after 6 weeks fungus development in the cambium with dark lines bounding the affected areas. Four trees were examined again after 14 months, by removing layers of tissue in succession. It should be remarked here that the greystreaked area in the tissue adjacent to the cambium is extremely shallow, and it is difficult to chop away the bark without also removing this. All trees showed the typical eye-shaped lesions with a black boundary in the bark, traces of yellow stain near the inoculation point, and grey-black streaks running up the wood. As far as could be judged the lengths of these were 5·25, 8·25, and 17 in. in the first three trees examined.

One of this group (inoculated in October 1951), which died in December 1952, was girdled at the points of inoculation by *Valsa*, which had also spread for considerable distances up and down the trunk.

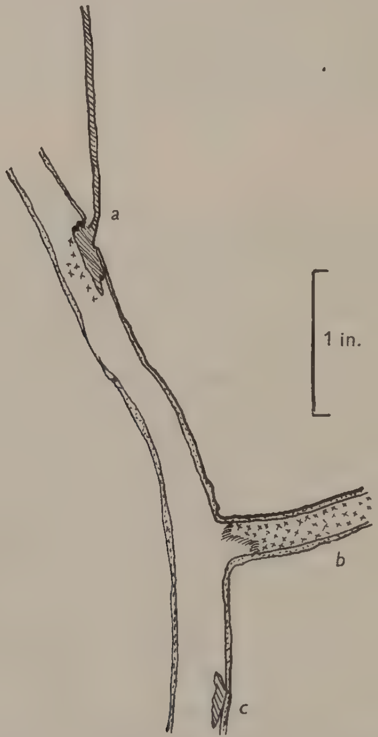
Experiment 5

The main roots of eight large mature trees were exposed for 2 ft. from the collar. Half-inch holes were drilled through the larger ones in several places, and in the collars at the bases of each main root to a depth of 2 in. A tightly fitting peg, made from yellow-stained, *Valsa*-infected wood, was driven into each of the 214 holes, and the roots were then re-covered with soil. The treated roots were up to 5 in. thick.

Eight months later, one root from each tree was removed and sawn longitudinally as shown in Text-fig. 5; from every peg invasion of the root tissues had occurred. The yellow-stained infected wood, bounded by the greyish black irregular line, and with occasional dark flecks in advance of the obviously stained portion, is exactly similar to what is to be observed when *Valsa* is acting as a wound parasite in a natural infection. In one root, where there were three closely spaced pegs, the black flecking extended over the whole 2 ft. sample, which was not long enough to show its limits. Radial and tangential spread of the fungus was much less than longitudinal. Mycelium was found in all parts of the yellow-stained zone, very numerous tyloses in the region of the dark boundary line, and occasional hyphae in the black flecks. Wood blocks, taken just in advance of the stain, and placed in moist chambers yielded *Valsa* in every instance.

Experiment 6

The experimental infections described above, although they have demonstrated that *Valsa* is pathogenic to the trunks and roots of mature trees, do not reproduce what we think to be the way in which sudden death itself may arise. In an attempt to achieve a, possibly, more natural method of inoculation, the entire surface root systems of a group of eight mature trees were dissected out by hand. This involved parts of the root systems of ten adjoining trees. As each few square feet of the root system was exposed, the soil was replaced by a heavy mulch of clove leaves, which was kept well watered. Eventually the whole of the upper soil was replaced by leaf-mould, in which profuse development of absorbing roots took place. Meanwhile, selected trees which had died from sudden death in another district were felled and brought to the site of the experiment where they were split into small billets, stacked, covered with coconut fronds, and kept damp. Profuse development of *Valsa* fructifications resulted, and the blocks were then placed on the surface of the leaf-mould, were themselves covered with litter, and were watered daily except during rainy periods. Thus, the absorbing roots were constantly exposed, over a long period, to infection by spores. The billets were renewed periodically as they became exhausted.



Text-fig. 6. Diagram of part of a root from one of the trees in Exp. 6. Stippling and shading indicate infection, and the way larger roots become infected from the smaller.

After 6 weeks, a sample of the roots was examined. Penetration of the finer roots by fungal hyphae could be seen, and in some of the fibrous roots hyphae could be found in the phloem and cambium. None could be seen in the xylem, but some had penetrated the endodermis. Two months later, *Valsa* was isolated from roots which were 2 mm. or more in diameter. After 6 months one of the larger roots showed decay in the distal portion and was brown, merging into intense yellow which continued to within 3 ft. of the collar. *Valsa* was recovered in culture. From subsequent observations it seems that the rate of development of fungus in this particular root was exceptionally rapid.

Fourteen months after the experiment began, the fibrous roots, over considerable areas, were dead. The attack was, however, not uniform, even in the same tree; some sectors of the root system being only slightly affected while others only a few feet away had no living fibrous roots. Generally speaking, the roots of the trees on the periphery of the block were less affected than those in the block itself. Text-fig. 6 shows that the larger roots become infected via the smaller ones and these, presumably, via the absorbing roots. The picture of invasion presented by these trees is not precisely similar to that in a naturally infected tree; in the experimental trees the attack is patchy, whereas in sudden death proper it seems to be more uniform. It seems possible that, in parts of the root system, the development of the disease in these experimental conditions is more rapid than it would be under natural conditions where the contact with spores is, presumably, not continuous.

Discussion

The evidence so far presented shows that *V. eugeniae* is invariably associated with, and can be isolated from, mature trees which are dying or have died from sudden death. It is also the only fungus consistently associated with slow decline in young trees. Although, for technical reasons, it is occasionally difficult to isolate it from these in pure culture, association between the fungus and the disease can always be established some little time after the death of the slow decline trees by the development of yellow stain spreading from the roots upwards and the *Valsa* fructifications which eventually develop.

No other fungus is consistently associated with either sudden death or slow decline; in fact, relatively few fungi have been observed on clove trees in the Zanzibar Protectorate. The dieback-causing pathogen, *Cryptosporella eugeniae*, is as common, perhaps more common, than *Valsa*. Various saprophytic fungi, mostly unidentified, occasionally occur on roots and trunks, one of the most frequent being *Kirschsteiniella* (Herb I.M.I. 44955*b*) which is often found in old trunk lesions, sometimes those caused by fire. A very common bark saprophyte is a species of *Pestalozia*. On decaying roots of both healthy and diseased trees a fungus, resembling a *Diplodia*, is frequent, particularly on the slowly decaying roots of slow decline trees. These are all obvious saprophytes or secondary invaders, but, in one plantation and for a few weeks only, clove seedlings were found being killed by a species of *Botryodiplodia*. On one occasion a young clove tree was found to be infected with *Armillaria*.

It might be argued that *Valsa* could be an ubiquitous fungus, which invades saprophytically most, if not all, dead trees. This cannot be maintained, because, apart from its role as a cause of dieback (where it is obviously acting as a primary pathogen) it is found only in clove trees which have died from sudden death or slow decline, and, not on those which have died of other causes; furthermore, we have never found it on any tree other than the clove.

The experimental evidence, which is in complete accord with field observations, shows that *Valsa* is a primary parasite of mature clove trees, that saplings are highly resistant to it (although the fungus can become established and grow slowly in their branches), and that seedlings are immune. It has also been established experimentally that the fungus can invade the roots of mature trees by means of spores washed from the surface of the soil.

We consider that the evidence, namely, invariable association with one particular fungus, the pathogenicity of which has been experimentally established; the absence of any other pathogen; and the demonstration that clove roots can be invaded by water-borne spores, make it probable that the sudden-death disease is caused by the destruction of the roots by *V. eugeniae*. It is necessary to consider how such an attack could take place, and how new outbreaks of disease might arise.

In this connexion, although *Valsa* as a cause of dieback is of minor economic importance compared with *Cryptosporella*, its occurrence in this form may be of great pathological significance. It occurs thus in areas which are still free from sudden death and we have found, in such areas, trees with large *Valsa* lesions in their trunks and producing perithecia freely. When such a tree eventually becomes girdled and dies, the fungus spreads and fructifies all over its surface, producing, eventually, a source of inoculum equivalent to a tree which has died from sudden death. This may well be the way in which a new outbreak arises.

It has already been described how it is usual in Zanzibar, and common in Pemba, to leave dead trees standing in the plantations. There are hundreds of acres of such trees in both islands, particularly in Zanzibar, which continue for many years to produce *Valsa* perithecia freely on their trunks and branches. Pycnidia, too, are produced in enormous numbers on the exposed surfaces of the stumps of felled trees, but they are evanescent and probably form a comparatively minor and local source of inoculum.

It is clear that sudden death arises in the root system, almost certainly in the absorbing roots. After rain, enormous quantities of ascospores and pycnospores are released and, as these are very small (averaging $4{\cdot}3 \times 1{\cdot}3\ \mu$ and $3{\cdot}4 \times 0{\cdot}8\ \mu$ respectively) they could, without difficulty, be washed into the soil and come in contact with the roots. In this connexion it has always been observed that the disease, in its most typical and rapidly spreading form, occurs in groves of mature trees in which the canopy is unbroken and where the ground is covered with leaf litter. In these conditions the absorbing roots are produced very near the surface, and thus are readily accessible. It is possible, too, that the innumerable and evenly distributed wounds caused by cultivation may facilitate invasion.

Although it has been shown experimentally that spores of *Valsa* can initiate invasion of the feeding roots of mature trees it is not clear how such an attack, which demonstrably takes a long time, can result in the typical sudden and spectacular collapse of a mature tree, rather than in a more gradual form of death such as is associated with slow decline. The explanation may possibly lie, in part, in the unusual drought resistance of the plant.

The clove tree is remarkable in that it can appear perfectly healthy when a large part of its root system has been removed or is non-functional. Storey, for example, severed all the laterals of a clove tree to a depth of 1·5 ft. at a distance of 4 ft. from the trunk without killing it. Campbell (1940) repeated this, but increased the depth of the trench to 2 ft., with the same result. Nutman & Roberts (1952), in a pruning experiment, have removed up to two-thirds of the main root systems of mature trees, which without exception showed no ill effects. Text-fig. 3*b* shows a tree with practically the whole of the root system dead and decayed, and on which no absorbing roots could be found; this tree was still alive.

It would be possible, therefore, for the fungus to be present in the root system for some time, and to reduce its efficiency greatly, without causing any visible symptoms. That invasion does, in fact, occur without overt symptoms is shown by the fact that *Valsa* can be isolated from the roots of still apparently healthy trees in areas where sudden death is epidemic. It must be emphasized, however, that it is only in these epidemic areas, where it is certain that the whole stand will shortly succumb, that this can be done. *Valsa* can never be isolated from the roots of trees in healthy areas.

It is possible that the attack on the absorbing system might be local, and the roots be attacked piecemeal, collapse taking place when the last remaining portions become non-functional. This does not seem likely, since examination of the roots of dying trees shows an apparently very even attack. We think it more likely that death results as a consequence of successive waves of attack on the absorbing roots, each wave establishing foci of infection in some of the fibrous roots. Eventually, when the whole of the fibrous system is fully impregnated, production of new absorbing roots must cease, and the tree would die, suddenly, particularly when dry conditions succeed the rains.

Observations on the experiment where spores have been applied to uninjured roots shows that two successive waves of absorbing roots have been attacked and that invasion of the fibrous, and some of the larger roots, has resulted. In this experiment, however, the disease is progressing faster in some parts of the root systems than in others. The artificial nature of the treatment, and the very high local concentrations of inoculum which must have been achieved may, however, explain this.

We have no evidence that spread ever takes place by root contact. In certain instances, where a new outbreak of disease arises at a distance from any other, it is manifest that root contact cannot be responsible. Where, however, as is usual in Pemba, outbreaks show sharp peripheral spread, root contact may be responsible or contributory, but not necessarily so. Again, there is little evidence as to the length of time the fungus may remain viable in roots or stumps after the death of the tree, but it could not be demonstrated in the roots of one tree which was beginning to decay and which had probably been dead for several years. Some indication of duration of viability is shown by the continued production of perithecia on dead trees until the bark disintegrates, a process which, in one plantation, we know to have taken 8 years. Long-term experiments, designed to yield information on these points, have been planned.

Slow decline, like sudden death, is invariably associated with the same proved pathogen, *V. eugeniae*, and is also associated with plantations which have been established on areas previously devastated by sudden death. With this disease, however, instead of a more or less uniform degeneration of the feeding roots and fibrous roots, a slow and progressive root-rot of the main laterals is the obvious cause of death.

Although the terms sudden death and slow decline have been used to describe two different conditions, yet there is, in fact, a complete gradation between the symptoms of the two diseases. Young saplings never exhibit typical sudden-death symptoms, but decline gradually over many years; somewhat older trees usually die rather more rapidly until, at about 20 years of age, a tree dying from slow decline might show symptoms very similar to those of sudden death; fully mature trees have never been observed to die as slowly as do young ones, but, even in these, dying may sometimes be a process which takes several months. Decay of the main lateral roots, however, is never seen in sudden death of mature trees.

We conclude that slow decline is a disease caused by attack by *Valsa* on the roots of trees which still retain some measure of juvenile resistance to the fungus, and thus might be considered to be the young-tree form of the sudden-death disease.

It is evident that 8- to 10-year-old trees, affected with slow decline, have certainly been suffering from progressive root-rot for some years, and must, therefore, have been infected at a comparatively early age. In most plantations in Zanzibar the young trees are exposed to infection at the time of planting, and for many years after this, either from infected and as yet undecayed roots of dead trees, or from spores from standing dead trees or stumps (Pl. 4*c*). No information is available as to whether the fungus could survive in the soil in the absence of dead clove roots, but this seems unlikely.

It may be that the roots of the young replants become infected at an early age, and that the fungus does not develop to any great extent until the juvenile resistance of the seedling begins to break down. On the other hand, although it would seem less probable, infection may not take place at all until this time. These questions are of great importance in connexion with the replanting of areas devastated by sudden death, and only long-term experiments will give the answers.

Control possibilities

Our knowledge is not yet adequate for definite control measures to be proposed. Only long-term experiments can supply this deficiency. It is, however, possible to say that any action which would result in a considerable reduction of the total amount of inoculum present in the plantations should slow down the progress of sudden death. The current practice of leaving dead and dying trees standing and, often, replanting young trees beneath them, is thoroughly unsound, and should be abandoned forthwith. Long-term experiments have been planned to investigate the effects of such treatments as the removal of all dead and dying trees, the cutting of isolation trenches, and so on, but results, unfortunately, are not likely to be available for practical use for some years.

As has been pointed out previously, new outbreaks probably arise as a consequence of uncontrolled dieback, which permits a high local concentration of *Valsa* inoculum to arise. It seems wise, therefore, to control dieback, not only because it is a damaging disease itself, but because of its importance in relation to sudden death. This presents great practical difficulties. Most dieback is caused by *Cryptosporella*, and only a comparatively small amount by *Valsa*. It is not possible to control the one without controlling the other, and this is a task, not only of considerable magnitude, but one which, owing to the apathy and conservatism of the local landowners, is likely to be opposed by them.

Slow decline presents a problem of another nature, and it seems unlikely that many of the considerable number of young trees planted in infected areas will reach maturity. It is not unlikely, however, that new plantings will succeed if, before they are made, all dead and dying trees are removed, all roots dug up, and the area devoted to the growing of some other crop for a few years. It is significant that some years ago in an attempt to control termites, one old infected area was dug over and all roots were removed before the new seedlings were set out. This plantation, although now of an age when slow decline might be expected already to have affected some of the stand, is, so far, completely healthy.

We have great pleasure in acknowledging the very great help which we have received from Dr R. M. Nattrass, Senior Pathologist of the Kenya Department of Agriculture, and from Dr H. H. Storey, C.M.G., F.R.S., who have both visited Zanzibar on several occasions and whose interest, encouragement, and helpful criticism have been unfailing. We also wish to thank Mr T. E. C. Robins, of the staff of the Clove Research Scheme, for his valuable practical assistance with the field work.

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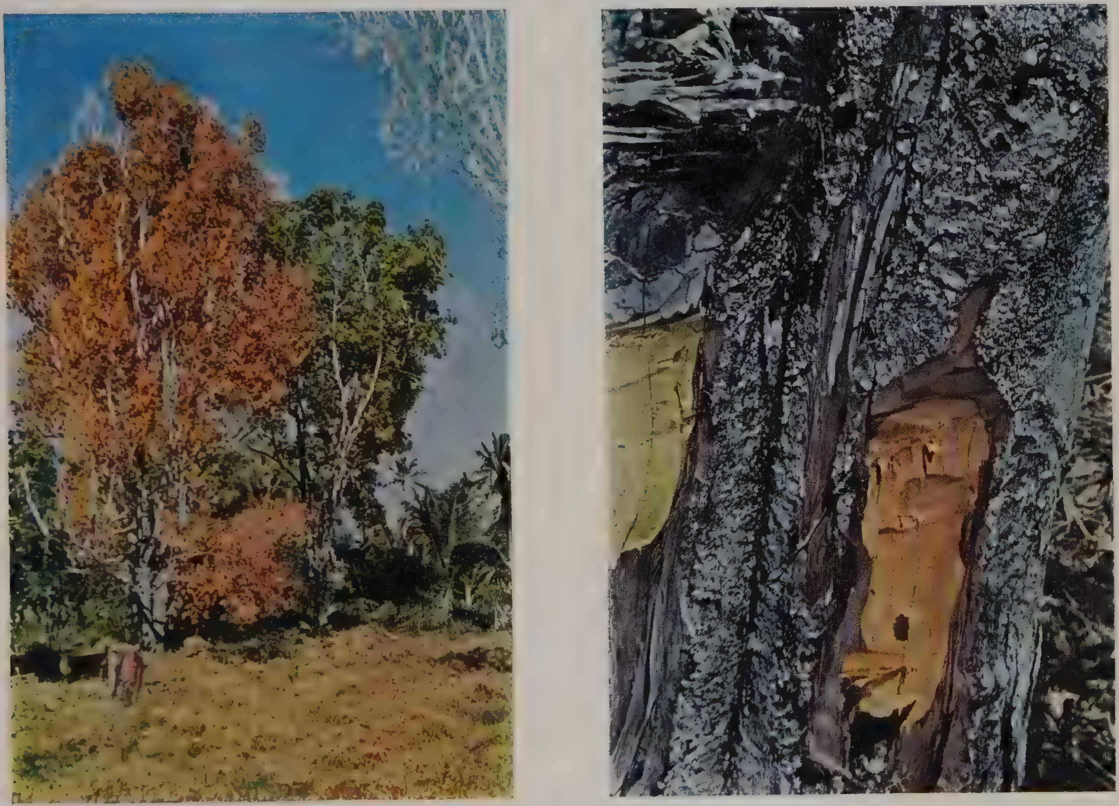
EXPLANATION OF PLATES 3 AND 4

PLATE 3 (in colour)

(*a*) Mature tree which had died of sudden death a few days previously.
(*b*) Trunk of sudden death tree slashed to expose the yellow stain caused by *Valsa*.

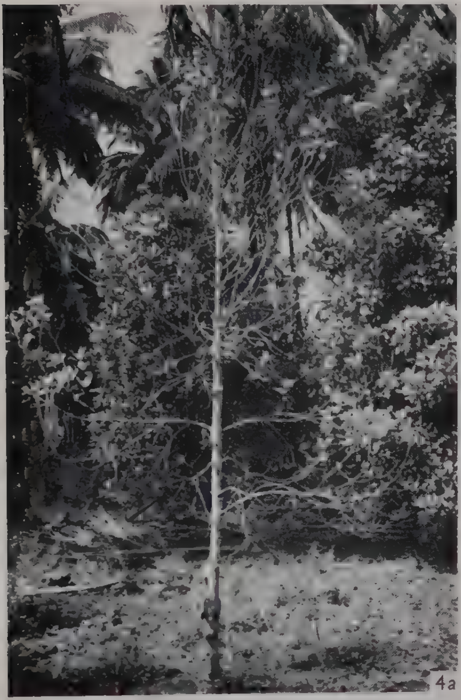
PLATE 4

(*a*) Young tree with slow decline.
(b_1) A point where *Valsa* had been inserted under the bark 21 days previously. The outer bark has been removed.
(b_2) As above, but cambium exposed. The dark streaks show the extent (9–10 in.) of the spread of the fungus.
(*c*) Seedling planted next to stump of tree which had recently died of sudden death. A common practice in Zanzibar Island is to leave the whole dead tree standing to provide shade for the replant.



a *b*

Nutman and Roberts—Valsa eugeniae *and sudden-death disease of clove tree*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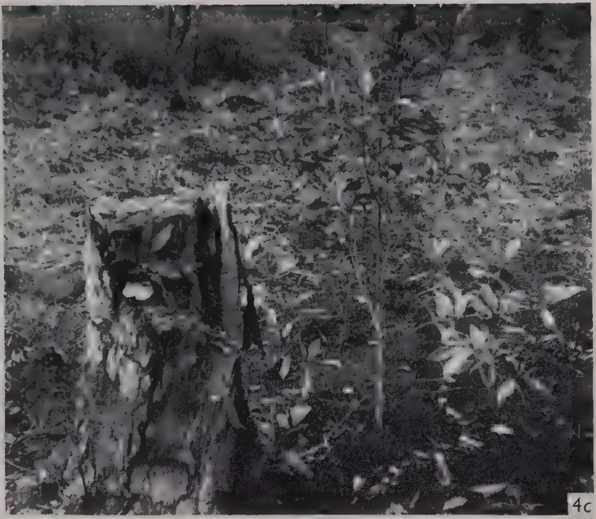
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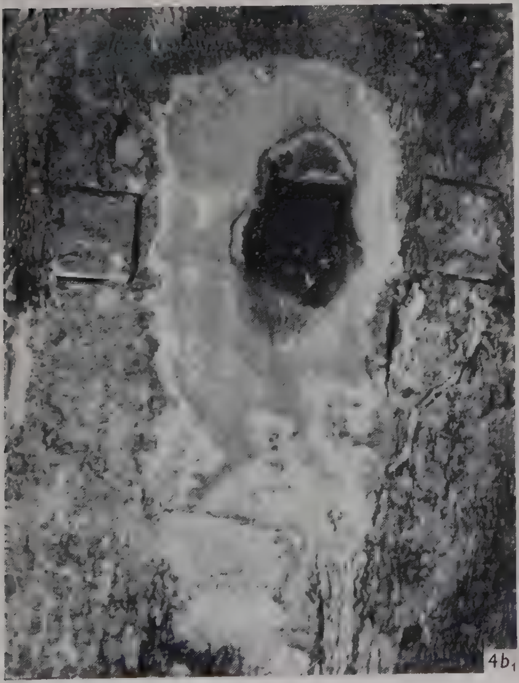
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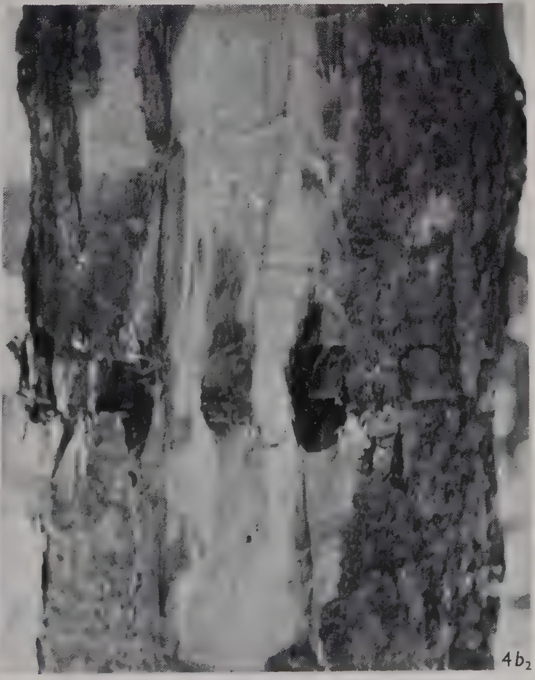
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NUTMAN & ROBERTS—Valsa eugeniae *and sudden-death disease of the clove tree*

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A MICRO-DROP APPLICATOR AND ITS USE FOR THE TREATMENT OF CERTAIN SMALL INSECTS WITH LIQUID INSECTICIDE

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(With Plates 5 and 6, and 3 Text-figures)

An instrument has been constructed for applying minute drops of liquid insecticide to selected parts of individual insects. The principle of the instrument, which is not original, is to express minute amounts of liquid from a hypodermic syringe, and blow the drop from the needle tip by a puff of air. This model is considered to have advantages over previous models working on the same principle. In particular, a very convenient form of fitting was developed for blowing the drop from the needle tip, and fittings of this type could be incorporated in existing similar applicators with little modification of the latter. The high directional accuracy of dropfire necessary for treating selected parts of small insects was achieved.

An immersion method was developed for determining the sizes of individual drops of Shell oil P31 delivered, and hence the uncontrolled variability of drop-volume. It showed that the standard deviation in the volumes of drops intended to be equal in size was about 9% of a mean volume of 0·017 μl. falling progressively to about 2% as the mean volume increased to 0·33 μl. These standard deviations, however, are of limited value, because the sizes of successive drops delivered appeared frequently to be correlated. A method in which the drops are delivered on to an oleophobic surface for measurement of the diameters of the oil lenses formed was found insufficiently accurate for determining the relative variability of drops of P31.

A suction method was devised for manipulating, without anaesthetization or chilling, small beetles such as *Calandra* and *Tribolium*. This enabled large numbers to be individually treated with the applicator in a reasonable time. A similar but slower method was used successfully with the larvae of *Ephestia*. The biological results achieved have been very satisfactory.

The effects of uncontrolled variation in drop size on dose-response data are discussed.

INTRODUCTION

A wide variety of laboratory methods are used in investigations upon contact insecticides, and techniques in which minute, measured amounts of liquid insecticide are applied to individual insects have long been recognized as valuable in complementing others. Such techniques of topical application permit investigations beyond the scope of those in which insects of a batch are treated *en masse*: in particular, the dependence of toxicity on locus of application can be studied, and the dose can be measured in absolute, as opposed to merely relative, units. The technique to be described here was developed for topical application of insecticides to certain stored product insects, in order to complement the film and direct spray techniques described respectively by Parkin & Green (1943) and Hewlett (1947). An essential requirement was that it should be suitable for the application of solutions of insecticide in medium-heavy base oils (e.g. Shell oil P31) of the type used for sprays against stored product insects in this country; they are much more viscous than spray base-oils such as odourless kerosene.

A rapid technique of application was desired, whereby the volume applied could be varied readily, but such that the uncontrolled variation of dose would be small. Random errors of dosage, the effects of which are often ignored, reduce the change in response produced by a change in dose, and may also introduce heterogeneity. The effects of these errors are further considered later.

Methods of topical application previously used are various. One in which liquid is taken up on a fine wire loop (see Wilson, 1949) has the disadvantage that the volume cannot readily be varied, since one loop is required for each level of volume, and should probably be calibrated for each solution used. A number of micro-pipettes have been built for topical application of insecticides, and the normal minimum quantity applicable with the earlier models (see Richardson, 1943) seems to have been 0·5–1·0 μl. Kerr (1951) used a form which would deliver 0·15 μl., and probably less. Dresden & Oppenoorth (1953) employed constriction pipettes to deliver about 0·03 μl. of acetone solutions, which is a dose of a suitable order for application to the smaller stored product insects. Shell oil P31, however, is too viscous to be delivered satisfactorily from fine capillary pipettes. Buck (1949) has described a micro-syringe suitable for placing liquid on insects. The uncontrolled variation of the individual doses applied to insects with these different devices has received little attention: in any case, to determine this exactly may be difficult, since the touching of the liquid on to the insect may introduce variation different in magnitude from that occurring in a calibration method. Calibrating his syringe by a special method, Buck found that the coefficient of variation of doses was 8–14% for a mean dose of 0·14 μl., decreasing with increasing mean dose.

Fisher (1952) has described an elegant method for forming, and applying to flies, supersaturated droplets or crystalline beads of DDT. It would seem, however, to be restricted in scope, since discrete beads of the liquid insecticide must be formed on the surface of a non-miscible liquid, i.e. spreading must not occur.

An apparatus of a type which, it seemed, would be suitable in principle for the object in view had been built in the Ministry of Supply, Chemical Defence Experimental Station, Porton. Hopf (1951) has described briefly, and illustrated, an instrument that was an adaptation of the prototype built at Porton. The principle of these instruments is as follows: A hypodermic syringe of moderately fine bore, containing the liquid to be applied, is mounted rigidly with the needle pointing downwards. The plunger is depressed by a micrometer screw-head, which is turned through a reduction worm gear. Minute and readily varied quantities of fluid can therefore be expressed. The drop expressed, however, is much too small to fall unaided, and is blown downwards off the needle tip by a puff of air through a special fitting. The insect must be placed so that the drop will hit it on the part of its body selected for treatment, and to do this a point in the path of the drop is marked by the intersection of two light beams. The performance of Hopf's instrument appeared to be adequate, since for mean drop sizes of 0·05–0·40 μl. the coefficient of variation was about 5%.

An instrument working on the same principle, but very different from Hopf's in construction, has been built at the Pest Infestation Laboratory. Rigidity was the main object of its general design, and modifications have been introduced to improve the mechanism for firing the drop. It has proved highly satisfactory for the application of P 31 solutions, with which it has been used mainly, but it also works well with solutions in odourless kerosene. It could probably be modified for delivering drops of volatile liquids.

Previous instruments working on this principle have been used to treat large insects such as locusts. However, the instrument to be described was required to treat selected parts of much smaller insects such as *Tribolium* and *Calandra* beetles. In consequence a high directional accuracy in firing drops was a *sine qua non*, and much effort was expended in achieving this. Furthermore, it was necessary to develop special methods for expeditious individual handling of large numbers of small insects.

CONSTRUCTION AND WORKING OF THE APPARATUS

The apparatus as a whole consists of a number of units: (1) the applicator-proper, which delivers and fires the drop of fluid, and on which the lights are mounted; (2) an adjustable table for holding the target; (3) an air pump and reservoir; (4) a unit for regulating the air flows and producing the firing puff of air; and (5) a pair of pedals, one operating a switch for firing the drop and the other cutting off suction during the handling of the insects. Pl. 5, fig. 1, is a general view of the apparatus.

General construction of the applicator

Pl. 6, fig. 1, illustrates the applicator-proper. The operative parts, other than the lights, are mounted on a heavy brass back-plate, which can be moved up and down on a dovetail slide fixed to a horseshoe foot of light alloy. The back-plate is locked in position by two screws acting on a spring-loaded gib-strip. The universal mountings of the lights slide up and down on two vertical rods: the top of each rod is held in a bracket on the back-plate, and the bottom in a guide attached to the horseshoe foot.

Delivery of the fluid

Syringe barrel

The fluid is held in the barrel of a micrometer syringe of a type supplied commercially. The bore is approximately 5 mm. The barrel is fixed in two hinged clamps lined with rubber, each locked by a nut on a hinged screw. A shoulder at the lower end of the barrel rests on a U-shaped bracket that takes the downward thrust on the syringe barrel; so that the clamps need not, and should not, be locked to grip the barrel tightly. This bracket is held on a rod projecting from the lower end of the back-plate.

A helical spring round the free portion of the plunger keeps the top of the plunger in contact with the micrometer head.

Micrometer head

This is of the type supplied with the syringe. One turn depresses the head 0·5 mm.

Reduction gear, etc.

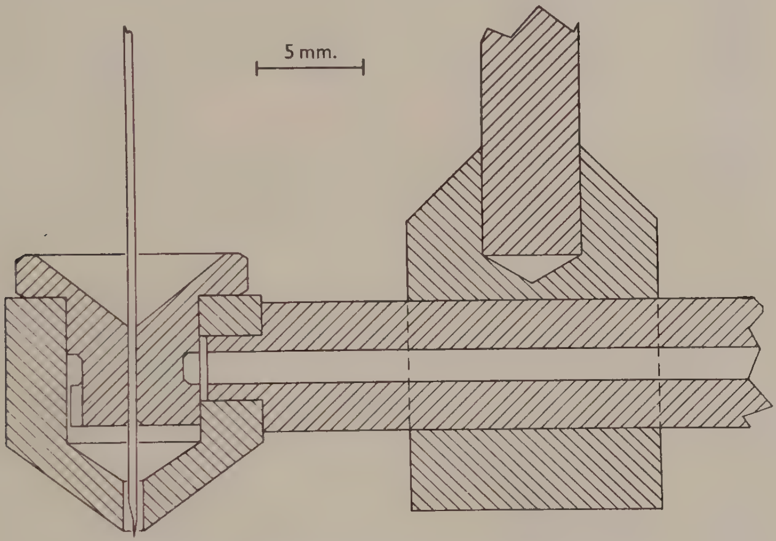
A keyed steel shaft on the top of the rotary portion of the micrometer head passes through a worm-wheel with a corresponding key-way. The shaft of the worm turning the worm-wheel bears a graduated drum. The ratio of the worm gear is 6 : 1. The worm mounting can be swung aside (see Pl. 6, fig. 2) to disengage it from the worm-wheel, so that the micrometer head can be turned directly when returning it to the top of its traverse. Spring-loaded plungers return the worm mounting to the engaged position, where it is locked by a screw.

After the original completion of the instrument a device was added to enable deliveries of up to 0·1 μl. to be repeated semi-automatically, that is without reading from the drum. It consists of a radial plunger (see Pl. 6, fig. 1) tipped with a thin polythene shoe, which, on depressing the plunger, grips the knurled edge of the graduated drum. The plunger is depressed and turned about the centre of the drum until it comes against a stop, when pressure is released and it is returned to its 'zero' position. The stop is movable, and its position determines how much fluid is delivered. The plunger is so constructed that the pressure applied to the edge of the drum is limited. A simpler mechanism could have been incorporated if it had been included in the original design.

Firing of the drop

As already explained, the drop is fired by directing a puff of air downwards around the tip of a hypodermic needle attached to the syringe. In previous models the puff of air has been a sudden surge rising from zero. In this model, on the other hand, sufficient air is kept flowing continuously to form the drop on the tip of the needle, and a sudden additional surge releases it. This arrangement, it has been found, discourages shattering of the drop on firing and produces greater directional repeatability of fire. It does, however, necessitate a slightly more elaborate mechanism for controlling the air flow.

In previous micro-drop applicators the shaft of the hypodermic needle passes through a hollow metal block. It enters it above through a hole in which it is a sliding fit, and emerges below through a hole somewhat larger than its external diameter. The puff of air is fed into the hollow block from the side, leaving it by the annular space around the emergent needle, so blowing the drop off the end of the needle. Three fittings were made for the instrument: (1) a first air block, very similar to those used by Lane (1947) and Levvy (1947) in instruments for producing streams of droplets; (2) an improved form of air block; and (3) what is called, for want of a better term, a 'jet-needle'. The last fitting proved to be the best. As mentioned before, directional repeatability of fire was essential in the application of drops to selected parts of small insects, and this requirement largely guided the development of the two latter fittings.



Text-fig. 1. Half-section of the improved air block. The needle blade is not shown in section.

First air block

In this block the lower surface, through which the needle emerged, was flat. The hollow, horizontal stem bearing the block was circular in cross-section, and the mounting such that the block was free to tilt from side to side during adjustment, which was therefore tedious. Experiments with this block led to the adoption of the continuous flow of air for forming the drop on the needle tip. The directional repeatability of fire achieved with this block was very poor, but efforts towards improvement were hampered by the difficulty of adjustment.

Improved air block

This consisted of the same parts as the first block, but (1) the needle emerged through a hole at the apex of a cone, in the hope of obtaining smoother air flow, and (2) the hollow stem bearing the block was made square in section, so that the block could be moved horizontally without being able to tilt. Text-fig. 1 is a vertical section of the improved block, and the block can also be seen in Pl. 5, fig. 2. Like those used by Lane and Levvy, and the first block made for the present instrument, the improved block was composite, consisting of a body and of a circular plug, through which the needle enters. The plug of the improved block was turned with an external channel opposite the air inlet. Three vertical channels were milled at equal intervals round the circumference of the plug, leading down from the turned channel, with the object of encouraging a radially symmetrical flow of air round the needle tip. The hole in the block through which the needle protrudes was 1 mm. in diameter. The block in Hopf's instrument is made in one piece, but a block of two parts—a plug fitting into a body—was convenient in so far as plugs pierced by holes of different sizes were easily made, enabling needles of different sizes to be tried.

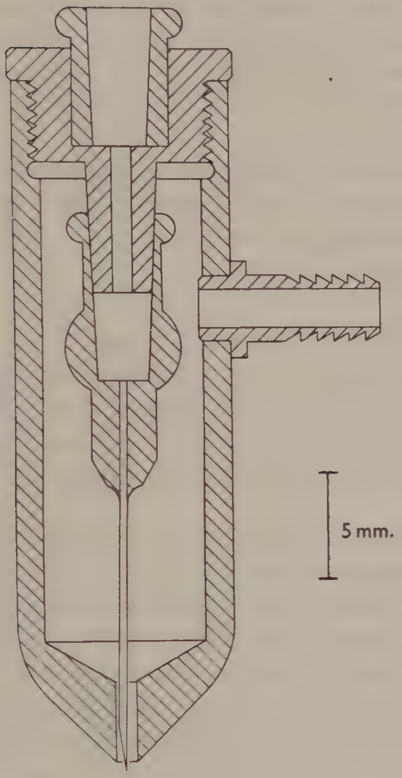
Making the diameter of the hole in the plug about 0·03 mm. over-size permitted centring adjustments on the needle tip, i.e. horizontal movement of the point relative to the axis of the hole through which the needle protruded, by horizontal movement of the air block. The joints between needle and plug, and between plug and body, were sealed by small amounts of oil.

A protracted series of trials was carried out with dyed P31, varying (1) the distance of protrusion of the needle tip below the air block, (2) the type of needle tip, (3) the centring adjustment of the needle point, and (4) the air flows. It was evident from the trials that all four factors were important in influencing the directional repeatability: for most combinations of the four factors this repeatability was bad. For good repeatability it was found that: (1) the needle point should protrude less than 1 mm. below the air block, and was best 0·4–0·6 mm. below; (2) a modified tip is much better than the standard tip supplied by the makers (details are given below); (3) the needle point should be centred; and (4) the total air flow and the surge of air should be the minimum consistent with prompt firing of the drop.

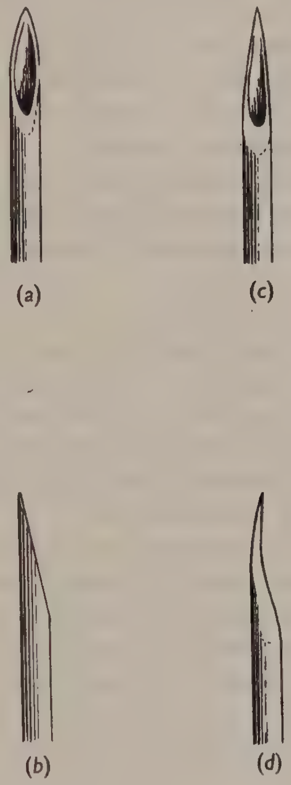
When sufficient directional repeatability had been achieved, the instrument was calibrated (for details, see below), and the uncontrolled variation between the volumes of individual drops was found not to be excessive. When, however, an attempt was made to treat respective batches of insects with drops of several different solutions during one day, changing from one solution to another was found to take too long, because the needle protrusion and centring adjustments had to be made at each change. To overcome the difficulty the jet-needle was constructed, and this has functioned as satisfactorily as the needle and air block.

Jet-needle

The effect of the jet-needle is to combine into one unit the functions of the air block and hypodermic needle, fixed at their best setting. Text-fig. 2 shows the jet-needle in section. Unlike the air block, the jet-needle is a press-fit on to the taper of the glass syringe barrel. It consists of two machined parts and a modified standard hypodermic needle. The upper part bears an internal taper above, into which the glass taper fits, and an external taper below, on to which the needle fits: both tapers are 'Record' fitting. The lower part is a cap screwing on to the upper, bearing a side tube for entry of air. The blade of the needle passes through a hole 1 mm. in diameter at the lower end of the cap, and its point protrudes 0·5 mm. As Text-fig. 2 indicates, the upper part is composite. To avoid making a special lathe tool, the internal taper was obtained by soldering a portion of the hilt of a hypodermic needle into a recess in the turning bearing the external taper.



Text-fig. 2.



Text-fig. 3.

Text-fig. 2. Half-section of the jet-needle. The needle blade is not shown in section.

Text-fig. 3. Tips of modified hypodermic needles, for use with the air block, with standard needles for comparison. The shafts are 0·4 mm. in diameter. (*a*), (*b*), standard; (*c*), (*d*), modified. The needle used with the jet-needle is similarly modified, except that the tip is unbent.

The point of the needle was centred by bending the blade at the level at which it leaves the hilt. This involved a tedious process of trial and error, but the adjustment, once achieved, is permanent.

Air is fed to the jet-needle through thin-walled rubber tubing. The tubing should be very flexible, so as to avoid strain on the glass barrel. A guide attached to the back plate holds the tubing (see Pl. 5, fig. 1), so that an accidental pull on the tubing is less likely to break the barrel, though the tubing can be pulled through the guide when required.

The directional repeatability of fire attainable with the jet-needle is very satisfactory, for drops of a given size do not deviate by more than about 30′ of arc from their mean path. The mean path varies slightly with the drop-size, and for smaller drops (say 0·05 μl.) deviates by about 15° from the vertical. This deviation is of little consequence, since repeatability is what matters, but the lights must be re-set when the drop-size is changed.

The jet-needle, it will be observed, is attached directly to the glass barrel. It is therefore capable of being fitted to existing applicators of the same type without special modification of the latter, except, perhaps, in the air supply.

Hypodermic needles

The most satisfactory needles tried had blades 0·400 ± 0·025 mm. in external diameter and about 0·25 mm. in internal diameter. Those of certain serum needles in common use are of the same external diameter but of narrower bore (about 0·15 mm.). Such needles were unsatisfactory for P 31, since the drop formed tardily on the needle tip, indicating considerable back pressure, but they were not tried with less viscous liquids.

With the air blocks, needles with blades 2·5 cm. long were used. Modification of the tips supplied by the makers greatly improved the firing of the drops, as was mentioned above. The sides of the tip of a standard needle were ground on a flat carborundum stone under a low-power binocular microscope, and the tip was polished on a soap stone. The tip was then bent until the point lay in the axis of the blade. Text-fig. 3 illustrates the modifications. Thus, when the needle blade was inserted into the air block, and adjusted so that its point lay in the axis of the hole in the block through which the blade emerged, the blade and hole were co-axial.

For the jet-needle, needles supplied by the makers were cut down to the right length and the tips ground *ab initio*. A wire was inserted (to prevent blockage), and the shortened blade ground on the carborundum stone to give it a chamfer of about 10° (i.e. a tip about 2·2 mm. long). The sides of the chamfer were ground down, as in modification of standard tips, and the tip was polished. For some obscure reason, bending the tip was here found to be highly inimical to directional repeatability of fire. However, an unbent tip gave a completely satisfactory performance, provided the point lay in the axis of the hole in the cap through which the needle protruded (see Text-fig. 2). The hole and needle blade were therefore not co-axial.

A perfect needle tip seems to be essential to satisfactory working, and great care should be taken to avoid damaging it. The needle was inserted into the plug of the air block under a microscope. When fitting the cap to the jet-needle the needle was protected by sheathing it with a blade cut from a larger needle.

Air supply and regulation

A small variable speed, oscillating-diaphragm pump, powered by a $\frac{1}{150}$ h.p. electric motor, supplies filtered air. The maximum air requirement is about 1·5 l./min., and the pump is run at considerably below its maximum speed. To smooth the flow, the air from the pump passes through a reservoir of about 3 l. capacity.

As already explained, there is a continuous flow of air through the jet-needle or air block, an additional surge releasing the drop. The additional surge is obtained by momentarily closing a by-pass through which a certain proportion of the total air supplied by the pump normally escapes to waste. The by-pass is closed by means of a solenoid operated by a foot switch: this arrangement is convenient and results in a repeatable speed of closure. When the apparatus is properly adjusted the interval between pressure on the pedal and release of the drop is imperceptible.

The course of the air is as follows:

Pump→reservoir→flowmeter A→needle valve A
→flowmeter B→jet-needle or air block
↓
needle valve B→by-pass (closable)→waste

The needle valves, by-pass, and solenoid form a single unit situated between the flowmeters (see Pl. 5, fig. 1), which are of the simple manometric type. Flowmeter A registers the total air flow, which is controlled by needle valve A. Needle valve B controls the flow to waste, and flowmeter B registers the flow to the jet-needle or air block. The difference in the flows registered by the two flowmeters when the by-pass is open is a measure of the surge to the jet-needle or air block on its closure.

Different drop-sizes require different air flows, and the correct flows for a given size are easily found by trial on each occasion of use. The continuous flow through the jet-needle is sufficient to cause the drop to form on the needle tip: this is observed by means of a small concave mirror on a semi-universal friction mount. The mount is borne on the rod which also holds the U-shaped syringe-retaining bracket and guide for the jet-needle air tube.

Location of the target

Lights

In each light a 6 V. bulb is used with a condenser lens, stop, and focusing lens to form an image of an aperture in the stop in the path of the falling drop. In one the aperture is a horizontal slit 0·25 × 3·2 mm. and in the other a hole 0·38 mm. in diameter. The stop and image are 7·5 cm. on either side of the focusing lens, but because of the inclination of the object to the optical axes of the lights, the images seen are slightly wider than the apertures. The lights are directed so that the images coincide 1·5–2·0 cm. below the needle point.

Adjustable table

The object to be treated is held on a table, the position of which can be varied by fine adjustments. The table (see Pl. 6, fig. 3) moves laterally by two screw adjustments, moves up and down on a rack and pinion, and rotates.

Setting up the apparatus

All solutions are dyed and filtered before use. The syringe barrel is filled, the jet-needle, with air flowing through it, is fitted, and sufficient liquid expelled to exclude all air, before the barrel with jet-needle attached is clamped into the applicator. The micrometer head is brought to bear on the top of the plunger, and, with no air flowing through the jet-needle, liquid is expelled from the barrel by turning the graduated drum through three or four revolutions. This floods with liquid the hole through which the needle protrudes from the jet-needle. The air flows are then slowly brought to about the levels suitable for firing test-drops of, say, 0·05 μl. The liquid so expelled on to the outside of the jet-needle is blotted away with a filter-paper. A series of about twenty test-drops is fired, during which the instrument settles down, and the air flows are then adjusted to the drop-size required. The flooding process has been found to lower considerably the air flows necessary for firing drops of a given size, though why is not clear. As pointed out earlier, low air flows help directional repeatability of fire. Occasionally the flooding has to be repeated before the desired result is achieved.

In order to set the lights, a drop is fired on to a filter-paper placed on the adjustable table, and the lights are adjusted until the beams intersect on the stain.

Changing from one solution to another, which involves dismantling and cleaning in addition to setting up, can be carried out in 10–15 min.

CALIBRATION

The object of the calibration was to determine (1) the mean volume of P31 oil delivered per division of the graduated drum, and (2) the relative variation between the sizes of individual drops (termed 'replicate drops') delivered by turning the drum a given number of divisions. A theoretical value for the mean volume per division could be obtained from the stated performance of the micrometer syringe barrel and the ratio of the worm gear, but an experimental determination was obviously desirable. However, the variation between replicate drops was more interesting, being a critical test of the performance of the instrument.

Knowing the density of P 31, the mean volume per division could be determined merely by finding the total weight of a large number of drops. But to determine the uncontrolled variation in drop-size was not so simple. The method used by Hopf is excellent in principle: he labelled the liquid with a radio-active material, and measured the radio-activity of single drops. A method of this sort could have been used, but radio-active materials are often troublesome in practice, requiring special apparatus and irksome precautions. It was therefore felt worthwhile to look for a method requiring ordinary facilities only.

The problem of measuring the drops delivered by the applicator was allied to that of determining the sizes of oil droplets produced by atomizing nozzles; suggesting that similar methods might be applicable. However, the drops from the applicator were on the whole much larger, and, it was to be expected, sets of replicate drops incomparably more uniform, than the oil droplets from most atomizing nozzles. In calibrating the applicator it was desired to measure with high precision the relative volumes of moderate numbers of drops; whereas in determining the particle size distributions of mists large numbers of droplets are usually measured, and high precision in the individual measurements is less important. In calibrating the applicator, systematic errors were naturally to be avoided, but random errors arising from the method of determination were also to be reduced to the utmost, because these would have increased the apparent variation between individual drops.

One type of method for drop-size determination depends upon inducing the drop to adopt some definite geometrical shape, linear measurement of which enables its volume to be calculated. In view of the ease and accuracy with which linear measurements can be made, such methods were clearly worth investigation. Anderson (1949) and de Jong (1950) used a simple method of this kind for determining the particle size distributions of oil mists. They allowed the droplets to fall on to a flat glass surface treated to make it oleophobic, and measured the diameters of the oil lenses so formed. The drop diameter was taken to be directly proportional to the diameter of the derived lens. For this assumption to be true, the curved surface of the lens must be spherical, and the contact angle between the oil and the oleophobic surface must always be the same; but a liquid-solid contact angle seems to be rather a variable quantity, and this method was not felt safe for calibrating the applicator, though it was included for comparison with another method. Attempts to introduce drops from the applicator into fine capillary tubes, and to measure the diameters of oil lenses formed from drops on liquid surfaces, were not very satisfactory. The method of Wilson (1949), in which drops are immersed in alcohol saturated with the oil, was found very difficult, since the drops sank too quickly. However, another immersion method was successfully developed.

A glass cell 2·5 cm. long × 1 cm. wide × 4 cm. high, with the pair of largest sides of clear, flat glass, is attached to a 3 × 1 in. glass slip by a rubber band. The cell is one-third filled with an immersion medium of glycerol containing 1 % by volume of Teepol (included because P 31 spreads on glycerol alone). Four to six drops of dyed P 31 are fired on to different points on the surface of the liquid in the cell, and each drop forms a lens. Each lens is immersed by gently delivering further medium on top of it from a wide-bore pipette fitted with a rubber teat. The spherical drop which forms is pushed down nearly to the bottom of the cell by the pipette from which medium is being slowly expelled. Further immersion medium is added until the cell is about three-quarters full. The glass slip with cell attached is clipped to the mechanical stage of a monocular microscope set with its tube horizontal. The horizontal diameter of each drop is measured by means of a filar micrometer eyepiece. The drops float upward, but at 20° C., the temperature at which the calibration was done, the viscosity of the immersion medium is sufficiently high to make the movement of the drops slow enough for satisfactory measurement. This method was used successfully on drops of up to 0·8 μl. in volume.

Though too slow for measuring very large numbers of drops, this immersion method, if carried out with care, is believed to be very accurate. By taking a number of readings on each of a few drops the micrometer setting was shown to be highly repeatable. Each reading was unbiased by the one before, since the hair-lines of the micrometer and the mechanical stage of the microscope were 'unset' between successive readings, necessitating the whole process of stage adjustment, focusing, and micrometer setting for each reading, with the micrometer scale invisible to the observer meanwhile. The setting could be repeated within one division of 500 or 600 on the micrometer. The relative error of determination in volume would be three times that in diameter, so that, provided the magnification was adjusted to give micrometer readings of over 300 divisions, the relative error in volume measurement could be expected to be not more than 1 %. No difference between the vertical and horizontal diameters of the drop was detected, nor any change in diameter as it floated up through the medium. A test showed that curvature of the field of the micrometer eyepiece was too little to cause an appreciable error. An advantage of the method was that the absolute, as well as the relative, volumes of drops could be determined, so that it achieved both objects of the calibration. However, the conversion to absolute units, in comparing stage and eyepiece micrometers, could introduce a further small error into the absolute, though not into the relative, determinations.

Three experimental methods in all were used in the calibration:

(1) The immersion method just described, for estimating both the mean volume delivered per drum division and the variation in drop volume.

(2) Weighing numbers of drops deposited on glass slips. A number of groups of drops were weighed separately, permitting a standard error for the determination to be calculated. After a pycnometric determination of the density of the dyed P 31 at 20° C., the weights were converted to volumes. This method estimated only the mean volume delivered per division.

(3) Measurement of the diameters of lenses derived from drops fired on to an oleophobic surface. Glass slips 3 × 1 in. were rendered oleophobic by the method of Anderson (1949), except that technical dimethyldichlorosilane was employed in

place of a mixture of methylchlorosilanes. Preliminary measurements indicated that the maximum diameter of a lens was usually 1–2 % greater than its minimum: in the determinations proper two diameters at right-angles were chosen at random, and the mean of the two taken. The focal lengths of the oil lenses were not determined, so this method estimated only the relative variation between replicate drops.

The needle and air block were fitted to the applicator during most of the calibration, but check determinations, by the immersion method only, were done with the jet-needle fitted. Table 1 gives the results. In the table, the figures for mean drop volumes have been expressed as the volume per 100 divisions of the drum, so that the figures for the different levels of drop-size are readily comparable.

Table 1 records standard errors and coefficients of variation, but these are of limited value. Each coefficient of variation by the lens method is considerably higher than the corresponding coefficient by the immersion method. This is taken to mean that appreciable errors arise from the lens method itself, and the original doubts of the method for the present purpose appear justified. There is no reason to suppose that the immersion method introduced comparable errors, but the value of the standard errors and coefficients of variation obtained with it is still restricted, because the sizes of drops delivered successively by the applicator were almost certainly not independent. For example, the micrometer readings for the diameters of the twenty successive 5-division drops fired with the jet-needle were 352, 349, 352, 347, 358, 357, 372, 350, 353, 353, 352, 351, 345, 349, 338, 340, 336, 345, 341 and 345. The 1st to the 4th, the 5th and 6th, and the 8th to the 12th fall very close to one another—too close, it seems, to occur by chance. The other series of replicate drops were similar. If the values of a variate, here the sizes of drops fired successively, are not independent, the ordinary statistical theory of errors breaks down. Thus, although the coefficients of variation from the immersion method may give a general idea of the magnitudes of the uncontrolled drop-size variations, it would be inadmissible to perform ordinary statistical tests on the data, such as t-tests, to discover whether the means of Table 1 differ significantly from the theoretical value of 1·667. In the results from the lens method, any non-independence was hidden by the superimposed errors of the method itself.

Generally, the variation between the volumes of replicate drops was satisfactorily small. Over the range of volume examined the relative variation evidently falls as the mean drop volume increases. The means for the volumes delivered per 100 divisions of the drum varied somewhat from one set of determinations to another. The weighted mean of the set of means is 1·686 μl., that is 1·18 % above the theoretical value of 1·667. The weighting of the means was carried out according to ordinary statistical theory, and the method of calculation is therefore open to objection, but is perhaps the best that can be done in the circumstances. Under many conditions no important error will be introduced by taking the mean volume actually delivered as equal to the theoretical.

Table 1. *The mean volume, and uncontrolled variation in volume, of drops of dyed P31 oil delivered by the micro-drop applicator*

Fitting		Method	No. of drops per determination	Number of drum divisions turned in delivering each drop					
				1	3	5 (1st trial)	5 (2nd trial)	10	20
Air block	Mean volume delivered in μl. per 100 divisions, ± S.E.	Weighing	150	—	—	—	—	—	1·725 ± 0·026
		Immersion	26	1·655 ± 0·030	1·782 ± 0·033	1·692 ± 0·020	1·690 ± 0·023	1·700 ± 0·018	1·678 ± 0·008
	Coefficient of variation of drop volume, %	Immersion	26	9·4	9·4	6·0	6·9	5·4	2·4
		Lens	26	23·1	—	11·1	9·9	—	—
Jet-needle	Mean volume delivered in μl. per 100 divisions, ± S.E.	Immersion	20	—	1·792 ± 0·034	1·737 ± 0·027	—	1·627 ± 0·017*	—
	Coefficient of variation of drop volume, %	Immersion	20	—	8·4	7·0	—	3·3*	

* Figures based on 10 drops.

MANIPULATION OF THE INSECTS

Special methods of manipulation are inevitably required for the rapid treatment of large numbers of small or moderately small insects by means of the micro-drop applicator. For flying insects anaesthetization or chilling at some stage will very often be unavoidable (see, for example, Fisher, 1952). However, both are probably best avoided if the insects are subsequently to be treated with insecticide, since they might influence the insects' response. A method avoiding anaesthetization and chilling has been devised, particularly for the manipulation of small beetles at 20° C. They are manipulated on the tips of suction tubes. Only weak suction (about 3 cm. of mercury), sufficient to support the weight of the insect, is required provided the suction tube is so applied that the insect is unable to use its muscular strength in attempting to free itself.

The effect of the technique is that each beetle is removed singly from a container holding a batch, treated with the applicator, and then transferred to a second container, where it remains for the observation period. For the most rapid treatment of large numbers of beetles the process is carried out by a team of two. Each operator has a mobile suction tube, held like a pencil. Operator A picks a beetle out of the first container with his suction tube, and transfers it to the tip of a static suction tube on the bench (see Pl. 6, fig. 4). Operator B, who sits in front of the applicator, uses his suction tube to transfer the beetle to another suction tube on the adjustable table (see Pl. 5, fig. 2). Viewing the beetle through the lens mounted on the table, B adjusts the table until (as shown by the lights) the part of the beetle selected for treatment is in the correct position, and fires the drop. He then picks up a small piece of filter-paper and brings it up to the treated beetle, which clings or walks on to it. B then quickly transfers the piece of paper with the insect on it to the second, observational container. Operator A removes the piece of paper from the container when the insect has walked off it. A suction tube cannot be applied directly to a treated insect, for fear of sucking some of the liquid from it, and hence the use of the piece of paper. When transferring the beetle from one suction tube to another, the suction to the first is cut off momentarily. B cuts off the suction from his mobile suction tube by pressure on a foot pedal: this allows him to keep his hand steady. A carries out the additional operations required when changing from one batch of beetles to the next. With practice, from six to ten batches each of twenty beetles can be treated per hour in this way, assuming no change of insecticidal solution.

One person alone can very well treat beetles, but not so quickly as the team of two. He transfers each beetle directly from the first container to the suction tube on the adjustable table, omitting the static tube. Removing the beetle from the first container is the slow stage here, but with the team of two, use of the static tube enables operator B to be steadily supplied with insects already arranged in the way most convenient for his manipulation.

The container for the beetles awaiting treatment is a metal ring standing on a piece of paper on a slide-ringing stage. The stage is turned to assist in the correct orientation of the beetle being picked up. Treated beetles are put within a glass ring standing on a filter-paper. Both the metal and the glass rings can be very lightly smeared with oil to prevent the escape of such beetles as *Calandra*.

The technique has been used very successfully with *Tribolium castaneum* Herbst., *T. confusum* Duv., *Calandra granaria* L., *C. oryzae* L. and *Alphitobius laevigatus* F. It must be emphasized, however, that for smooth and rapid working the details of the technique need to be carefully adjusted to the particular species used. For example, the paper on which the beetles are confined before treatment must be of the right kind: if too smooth a beetle cannot right itself if it falls over on to its back, but if too rough the beetle grips it with its claws, and is not picked up readily with a suction tube. A smoother paper is required for *Tribolium* than for *Calandra*. Again, the piece of paper presented to the treated beetle must be large enough to take the beetle but small enough to be picked up easily with the suction tube.

The suction tubes (see Pl. 6, figs. 3, 4) are particularly important. Though requiring care, the manufacture of the suction tip is simple: a piece of glass tubing is drawn out, bent, and cut, and the end on which the insect will be held is ground and flame-polished. It is convenient for each mobile suction tube to consist of a handle into which the tube bearing the suction tip is inserted. The same suction tubes could be used for *Tribolium* and *Calandra*, but tips of wider bore were required for *Alphitobius*.

A simplified but slower form of technique has been employed to treat the larvae of the moth *Ephestia elutella* Wlk. A length of the body near the site of dosage needs to be kept rigid, and to do this the larva is held by suction on a slit-shaped opening at the end of a tube (see Pl. 6, fig. 4). This form of suction tip is made merely by flattening the end of a piece of glass tubing. The larva is picked up with a mobile suction tube, and, without transfer to another tube, is held under the applicator for treatment. Transfer of a larva from one tube to another is not very satisfactory. The larva is located by hand. This technique, in which the insect is treated while held on the tube by which it is first picked up, was not suitable for the beetles. The beetles are more active than the moth larvae, so that, if a beetle is running freely, it is difficult to apply the suction tip in a position suitable to the subsequent treatment.

BIOLOGICAL RESULTS

The applicator is now in constant use, and the large quantity of biological results obtained has been very satisfactory, since the response has been well correlated with dose. Consider as an example an experiment in which *Tribolium castaneum* were treated with allethrin in Shell oil P31, in order to compare the effects for two sites of application. The sites of application were both dorsal, one at the junction of the head and prothorax and the other at the junction of the prothorax and mesothorax.

The allethrin solutions were made by dilution of a commercial concentrate of about 75 % purity. One drop of 0·05 μl. nearly, formed by turning the drum of the applicator three divisions, was fired on to each beetle. The insecticide spread somewhat after application, but was more localized at the posterior site, since here a higher proportion was drawn into the intersegmental fold. Immediately after treatment each batch of about twenty beetles was transferred to a room kept at 25° C. and 70 % relative humidity, where it remained during the observation period.

Table 2 gives the mortalities observed 6 days after treatment, at which time none of the 100 control beetles had died. The P31 alone had been shown previously to be non-toxic at the dose used. The following pair of parallel lines for regression of probit mortality (Y) on log-concentration (x) was computed:

$$\left.\begin{array}{ll}\text{Head-prothorax:} & Y=0{\cdot}09+7{\cdot}03x\\ \text{Pro-meso-thorax:} & Y=1{\cdot}27+7{\cdot}03x\end{array}\right\}\chi^2_{[3]}=2{\cdot}3,\ P=0{\cdot}52.$$

TABLE 2. *The toxicity to* Tribolium castaneum *of allethrin in Shell oil P31 applied topically at a rate of* 0·05 *μl. per beetle*

		Allethrin (commercial grade) concentration, %, w/v		
Site of application	Response 6 days after treatment as	2·50	3·53	5·00
Anterior*	Number dead	1/59	11/57	26/57
	Mortality, %	2	19	46
Posterior†	Number dead	10/60	31/60	54/59
	Mortality, %	17	52	92

* Dorsally, at the junction of head and prothorax.
† Dorsally, at the junction of prothorax and mesothorax.

The value of P indicates that the data could be satisfactorily represented by the pair of parallel lines. The standard error of the common slope is 0·78. It is estimated that the allethrin was 1·47 times as lethal at the posterior site of application as at the anterior; and that the 5 % limits of this relative potency are 1·51 and 1·65.

Possibly the insecticide was more toxic when applied at the posterior site of application because there it had easier access to the tracheal system. The liquid applied dorsally at the junction of the prothorax and mesothorax, since it ran into the intersegmental fold, readily reached the anterior pair of spiracles.

DISCUSSION

The glycerol-Teepol medium, used in determining the drop-size for P31, was satisfactory, but for the technique described above an even more viscous medium might be better, because it would further slow down the upward movement of the drops. However, since the determinations of Table 1 were done, it has been found possible, using the glycerol medium, to arrest the movement of the drops relative to the microscope. The drops are introduced into a special cell in which medium flows downward at a velocity adjusted to neutralize their flotation. Odourless kerosene spreads on the surface of the glycerol medium, and on those of others tried, and so measurements on drops of this oil were not carried out.

The consequences of random variation of drop-size on dose-response data have already been mentioned briefly. It is interesting to consider these in more detail, even though the considerations would not apply precisely to data obtained with the applicator, since the variation of the drops it gives is probably not random. The reduction of the change in response produced by a change in dose is an insidious effect, since it is not apparent from the dose-response data obtained. Finney (1952, pp. 170–2) has given the theory which enables this effect to be calculated for random variations in dose. Although the median effective dose would be unaltered, the slope of the probit-log-dose regression line would be reduced from β (if there were no errors of dosage) to $b=\beta/(1+\beta^2\gamma^2)^{\frac{1}{2}}$, where γ is the standard deviation of the dosage (in this context, of the log-volumes of the individual drops). β, being the reciprocal of the standard deviation of log-tolerances, is a purely biological quantity, whereas b is not. If C is the standard deviation of volume expressed as a percentage of the mean, γ is equal to $C/100 \log_e 10$, assuming the logarithms of the doses are to base 10. The values of b corresponding to different values of β and C can therefore be calculated, and this has been done in constructing Table 3.

TABLE 3. *The values of the expected slope, b, when the individual dose has a coefficient of variation of C*

	β				
C	2	4	6	8	10
5	2·00	3·98	5·95	7·88	9·77
10	1·99	3·94	5·81	7·56	9·17
15	1·98	3·87	5·59	7·09	8·38
20	1·97	3·78	5·32	6·57	7·55

It will be seen that the effects of the dosage errors are much more pronounced for higher values of β. For $C=10$, b will not be more than 10 % below β for $\beta \leqslant 10$, and so the performance of the applicator even for the smallest drops obtained (about 0·017 μl.) seems not unreasonable. Often the situation will not be exactly that just considered, in that solutions containing different concentrations of insecticide will be applied at one mean level of volume per insect, as in the experiment described in the preceding section of this paper. The relation between b and β, which is then different from that in the situation just dealt with, has also been examined by Finney.

In addition to influencing the slope of the probit-log-dose line, variation in individual dose must normally tend to introduce heterogeneity. This is because if the individual dose varies, it will rarely be possible to control the mean dose exactly. If the standard deviation of log-dose within a batch is γ, the mean log-dose given to n insects will be subject to a standard error of $\gamma/n^{\frac{1}{2}}$. General experience, however, suggests that the amount of heterogeneity introduced will usually be small unless γ is very high. For example, if $C=10$ and $n=50$, $\gamma/n^{\frac{1}{2}}=0{\cdot}006$, and therefore the errors in dosage will be equivalent to errors in the mean log-dose per batch of the order of 0·006.

The applicator works satisfactorily in delivering drops down to 0·017 μl. in volume, but improvement in its performance may well be possible. In particular, the occurrence of short series of replicate drops of nearly constant volume suggests that there may be some way of reducing the uncontrolled variation in drop-size. Clearly there is scope for experiment in the design of the jet-needle. Why flooding should reduce the air flows necessary for firing the drop is not clear, but it might do so because liquid afterwards remains caught in the narrow gap between the proximal end of the needle tip and the wall of the hole from which the point projects. If so, a hole of smaller diameter might be beneficial. In delivering a drop of 0·017 μl. the graduated drum is turned only one division, leading to possible errors from scale reading. A worm gear of perhaps 20 : 1 reduction, in place of 6 : 1, would probably be an improvement, and perhaps a syringe barrel of narrower bore. Other modifications purely for convenience of manufacture and working could also be made.* In general, the possibilities of the principle on which the instrument works appear to be by no means exhausted, and further development might permit closer control of the drop-size, together with the delivery of smaller drops.

The micro-drop applicator was designed jointly with Mr W. K. Cordaroy, by whom it was made in the Laboratory's workshops, and I would like to record my thanks to him for his co-operation. I also wish to thank Mr J. H. Hammond for advising on the optical system of the lights and for taking the photographs. This paper is published by permission of the Department of Scientific and Industrial Research.

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* An improved model has now been built.

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EXPLANATION OF PLATES 5 AND 6

PLATE 5

Fig. 1. General view of the apparatus, arranged for a left-handed person seated in front of the applicator-proper with a right-handed person seated on his left.

Fig. 2. A treated beetle, *Calandra granaria*, held on the tip of the suction tube on the adjustable table (without lens fitted). A piece of paper, held on a mobile suction tube, is being brought up underneath the beetle for the latter to cling to. The air block, with its mounting is also visible.

PLATE 6

Fig. 1. The applicator-proper.

Fig. 2. The reduction gear of the applicator-proper in the disengaged position.

Fig. 3. The adjustable table with mounted lens, and suction tube for holding the insect.

Fig. 4. A moth larva, *Ephestia elutella*, held on the tip of a suction tube.

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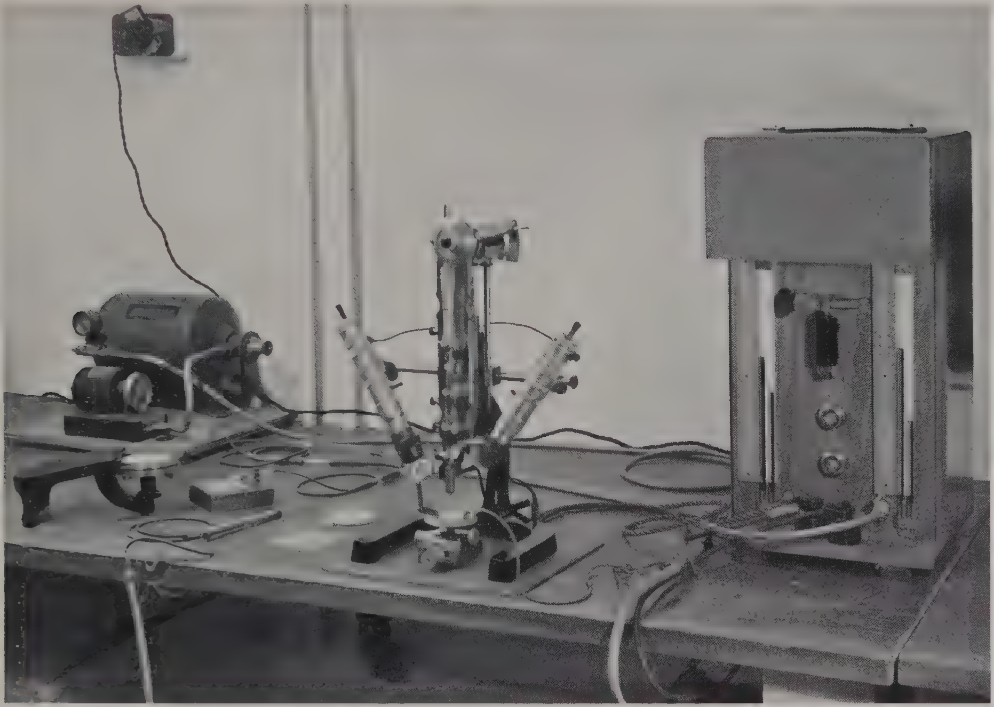


Fig. 1.

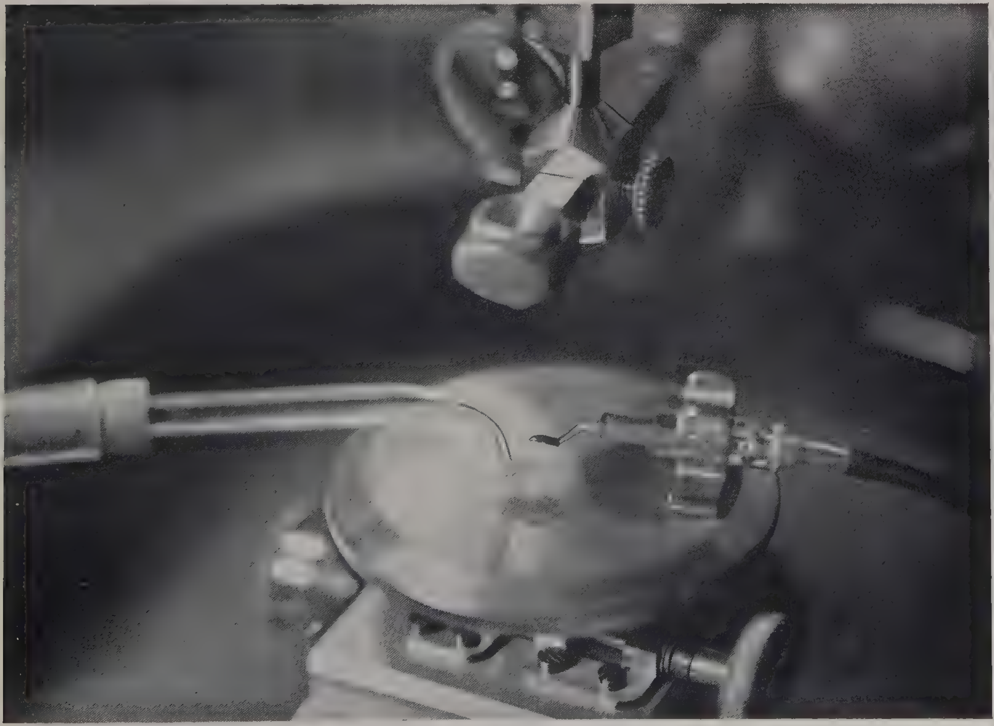
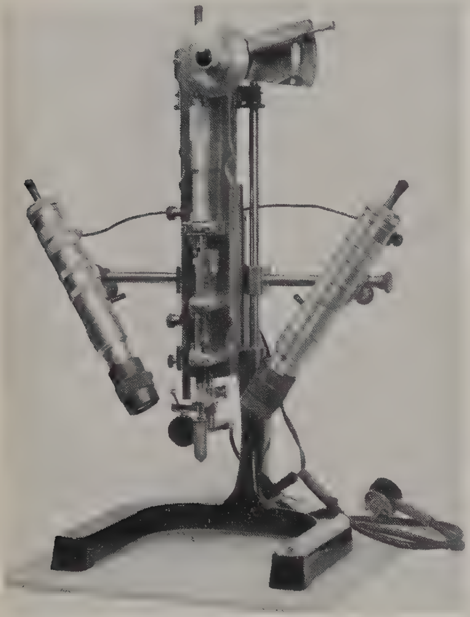


Fig. 2.

HEWLETT—*A micro-drop applicator*



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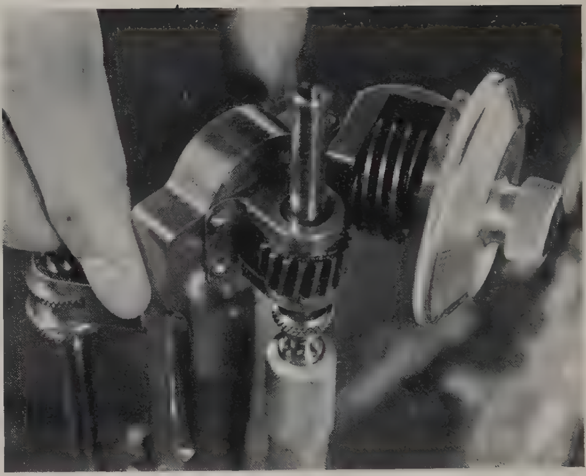

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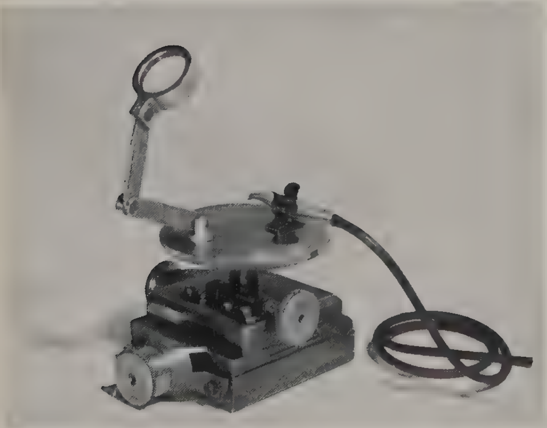

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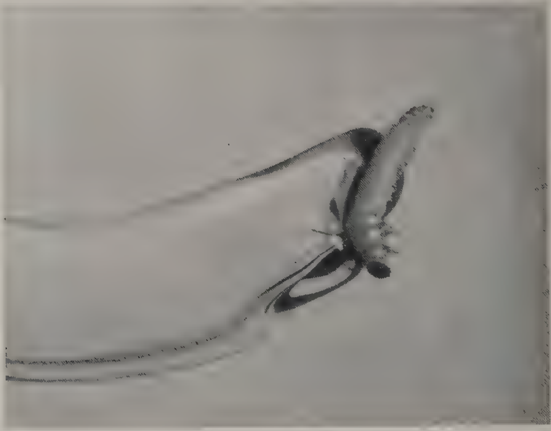

Fig. 4.

Hewlett—*A micro-drop applicator*

BRADBURY, F. R. & ARMSTRONG, G. (1954). *Ann. appl. Biol.* **41** (1), 65–76.

A METHOD FOR MEASURING THE NARCOTIC ACTION OF CHEMICALS USING THE GRAIN WEEVIL, *CALANDRA GRANARIA*

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(With Plate 7 and 1 Text-figure)

A method of measuring narcotic potency is described. Grain weevils (*Calandra granaria*) are exposed to the narcotic vapour in 2l. round-bottomed flasks illuminated from the bottom, and narcotic potency measured as number of grain weevils so affected as to be unable to climb the glass walls. Some common anaesthetics have been examined as an illustration of this method. Results are fitted by the probit plane

$$Y = A + b_1 \log \alpha + b_2 \log t,$$

where Y = a function of the number of insects affected (probit); α = the thermodynamic concentration of the narcotic vapour; t = time of exposure of insects to the narcotic vapour. The values of the coefficients b_1 and b_2 are discussed in relation to the biological action of the narcotic vapours.

INTRODUCTION

The narcotic properties of chemicals have been studied in this laboratory as an aspect of the problem of the mode of action of drugs on living organisms.

The narcotic action of chemicals is very convenient for a study of this problem because it is a mild and reversible effect eminently suited for an investigation of the thermodynamics of the biological action of chemicals. Even so, a study of the literature on narcotics reveals that the conflict between adsorption and solubility theories of narcotic action still persists after fifty years of intensive quantitative study.

Two obstacles to progress in this field have been the confusion arising from expressing potencies in terms of concentrations of chemical instead of partial saturations or thermodynamic concentrations, and the absence of a simple quantitative measure of narcotic action. The first difficulty has been removed by Ferguson (1939) who developed the clarifying concept of expressing biological potency on a scale of thermodynamic activity. It is the aim of this paper to describe a quantitative measurement of narcotic potency which may prove useful in further explorations of relationships between chemical structure and narcotic action.

The immediate effects of Ferguson's treatment were: (i) to point out that the correlation between any one set of physical properties, say, surface activity, and biological properties existed because the physical properties used by various theorists were themselves all related to the underlying thermodynamic activity; and (ii), to divide the biological effects of chemicals into the broad categories of chemical and physical. Thus Ferguson & Pirie (1948) describe drugs as exerting chemical action if they are effective at very low values of thermodynamic activity, and those toxic in a range from 0·1 to 1·0 (saturation) as working by a physical mechanism.

The introduction of a thermodynamic scale of comparison of biological potencies has reversed some classical concepts of the relationship between chemical structure and biological activity, such as, for example, the theory that biological activity increased during the ascent of a homologous series until the 'cut-off' was reached (see Ferguson & Pirie, 1948). It is therefore necessary to re-examine the effect of chemical structure on biological potency when this is measured on a scale of thermodynamic concentrations. In a recent publication Ferguson (1951) has recorded the narcotic potency to grain weevils of a number of aliphatic compounds including hydrocarbons, chlorohydrocarbons, ketones, nitriles, alcohols, ethers and nitroparaffins. In this paper we record the behaviour of some well-known anaesthetic compounds with the twin objects of illustrating the method of measurement and establishing standards to which the narcotic properties of other compounds could be referred.

METHOD

Ferguson (1951) used grain weevils as the biological organism in his narcosis experiments. The grain weevil has the advantage of being small and easily bred, and it can therefore be used in sufficient numbers to permit a reliable estimate of biological response by minimizing the error due to variation between individual organisms. We have followed Ferguson in using this insect as our biological material but we have replaced his qualitative description of degree of narcosis by a numerical measure. The grain weevil tends to move away from light (but see Richards, 1951), and also to climb the walls of a glass vessel. We have made use of these properties by confining the test insects in a glass vessel illuminated from the bottom. Under these conditions the grain weevils climb rapidly to the top of the vessel, but in the presence of a narcotic vapour the insects begin to lose their ability to respond to the stimuli of light and gravity and increasing numbers remain in the bottom of the vessel. We read the number so affected as an index of degree of narcosis. Essentially, therefore, the method is a graded response measured as an all or none effect (Clark, 1937*a*).

To facilitate comparative treatment of the results the percentage of insects narcotized or 'knocked down' in the experiments have been converted to probits.

In all our experiments we have calculated and recorded dosage rates in terms of thermodynamic concentrations. The chemicals were applied as vapours and under these conditions the thermodynamic concentration (or thermodynamic activity) of a compound is numerically equal to the relative saturation of the vapour, P_t/P_s, where P_t is the pressure of the vapour of the substance in the atmosphere surrounding the test insects and P_s is the saturated vapour pressure of the substance at the temperature of the experiment. Throughout this paper the expression 'thermodynamic concentration' is used to indicate the relative saturation of the vapour and is denoted by the symbol α. (For a full discussion of the thermodynamic principles involved in this treatment see Ferguson, 1939, 1951.)

Each chemical was tested at five thermodynamic concentrations, and observations of narcotic effect made over a period of 4 hr.

The thermodynamic concentrations and the times were chosen on a logarithmic scale. The intervals in $\log \alpha$ were 0·2; the values of α were selected from preliminary experiments so as to bring about a 50% effect (probit 5·0 referred to in this paper as 'ED 50') as near to the centre of the design as possible. The times at which measurements were made were approximately $\frac{1}{2}$, 1, 2 and 4 hr. from the beginning of the experiment. Time of exposure in hours is referred to as 't'.

The probits of narcotic action were plotted against $\log \alpha$ plus $\log t$ and treated by the probit-plane method of Finney (1952).

Normally the compounds were examined in groups of seven, each being tested in triplicate in a Youden square design. The object of this arrangement was to permit an adjustment of variation between days as described by Moore & Bliss (1942), but only rarely have we found the 'between day' variation to be significant compared with the variation between replicates, and adjustments have not usually been necessary.

As an example of the method we report here the results on six established anaesthetic compounds: ether, chloroform, *cyclo*propane, trichloroethylene, vinyl ether and trifluorodibromoethane $CF_3.CHBr_2$ (Robbins, 1946). A seventh, nitrous oxide, was included in the design but proved insufficiently potent below 2 atmospheres pressure (the maximum we can safely hold in our flasks) to provide any reliable estimate of its ED 50 to grain weevils.

EXPERIMENTAL PROCEDURE

Test insect

The test insect was the grain weevil, *Calandra granaria*, bred on wheat in a constant temperature humidity chamber maintained at 77 ± 3° F. and 66 ± 6% relative humidity. Weevils were taken for experiments between 6 and 8 weeks after the initiation of the culture.

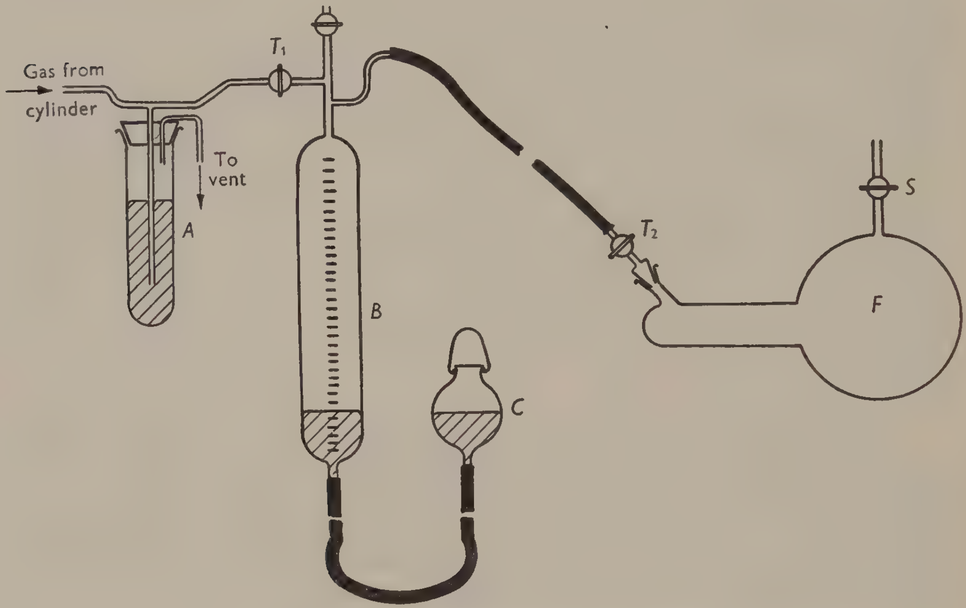
Apparatus

The insects were exposed to the narcotic in a 2 l. round-bottomed flask modified as shown in Text-fig. 1. A capillary side-arm cock was sealed on to one side of the bulb of the flask, the neck was closed and a B 14 socket and glass stopper sealed on so that it was in the plane of the capillary arm. For experiments with gases the stopper could be replaced by a glass tube and tap shown in the figure.

The volume of each flask was measured, and the appropriate volume of chemical needed to give the required thermodynamic concentration at 25° C. calculated from the expression

$$v = \frac{\alpha p_0 M v_f}{760 \times 24{\cdot}5 \rho} \text{ ml.,}$$

where α = the fractional saturation of the vapour (thermodynamic concentration), p_0 = saturated vapour pressure at 25° C., M = molecular weight, v_f = volume of the flask, and ρ = density of the liquid at 25° C. Vapour pressures and densities were taken from the literature where possible; some of them have been determined specially for this work. In the case of vinyl ether the vapour pressure used in the above expression was determined in the laboratory using the commercial anaesthetic grade material.



Text-fig. 1. Arrangement for testing gases.

With an Agla micrometer syringe the calculated volume of liquid was introduced into the neck of the flask, which was arranged so that the liquid did not run down into the bulb, and the stopper replaced. With the side-arm cock open the liquid was allowed to evaporate, if necessary with the aid of a gentle heat from a small bunsen flame. It was expected that the expanding vapour would thus expel an equal volume of air from the flask via the capillary side arm. When all the liquid had evaporated the side-arm cock was closed and the flask placed in the exposure apparatus. The partial pressures of narcotic vapour used in the present experiments were always so small as not to interfere seriously with the partial pressure of oxygen in the experimental flask, but in experiments with gases it is sometimes necessary to introduce some oxygen into the flask to maintain its normal partial pressure. When working with gases the arrangement shown in Text-fig. 1 was used. Gas from a cylinder was admitted to the graduated bulb *B*, via safety valve *A* and tap T_1. The measured volume was transferred from *B* to the evacuated flask *F* via T_2 and pressure finally restored to atmospheric by admitting air through the capillary arm *S*. When oxygen was required this was measured and admitted to the flask in the same way as the narcotic gas. By raising *C* positive pressure could be set up in *F*. Proper precautions were taken to prevent injury should the flask fail under pressure but it was never possible to work for long periods (4 hr.) with pressure greater than 2 atm. in this apparatus on account of leakage of gas.

The exposure apparatus is shown in Pl. 7. It consists of a stand with 6 in. diameter holes shaped to support the bowl of the flasks and provided with fluorescent lighting below with a celastoid sheet inserted to protect the flasks from heat. A Perspex sheet placed over the front protects the flasks from draught. Three such stands were employed and all were used in the constant temperature humidity room in which the grain weevils are cultured. Under these conditions the temperature inside the exposure boxes was kept at 25 ± 1° C.

Exposure of insects to the vapour

When the liquid had evaporated and the flask was at 25° C. twenty grain weevils, previously counted into a 3 × 1 in. specimen tube, were introduced whilst the stopper was removed and quickly replaced by a second operator.* The flask was then turned into the upright position with the neck vertical. The experiment was timed from the insertion of the grain weevils. Each stand contained five different doses of the chemical and one control without chemical.

In the control flasks the insects climbed up the walls of the flask away from the light, occasionally falling from the neck into the base of the flask and quickly making their way up the walls again. Normally the control would show only three to five out of twenty insects within the circle in the bottom of the flask outlined by the hole in the stand. The index of narcosis recorded for each flask was the number of insects to be found at any given time within this ring in the bottom of the flask. A group of such readings was taken at $\frac{1}{2}$, 1, 2 and 4 hr. from the beginning of the experiment. Each group consisted of five sets of readings taken at 5 min. intervals equally spaced round the appropriate time.

At the conclusion of the experiment the grain weevils were removed from the flask and placed in a specimen tube with a few grains of wheat and stored in the constant temperature humidity room for 72 hr.; the percentage mortality, if any, was then recorded. After each experiment the flasks were freed from chemical by washing with detergent and hot water, followed by thorough rinsing with hot water and drying at 120° C. In the earlier experiments chemical was removed from the flasks by washing with acetone but occasional large control values were traced to incomplete removal of acetone from the flasks and this method of cleaning was thereupon abandoned.

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* When working with gases under positive pressure it was necessary of course to insert the weevils before admitting the gases.

Purity of materials

Ether, chloroform, *cyclo*propane, and vinyl ether were the normal marketed anaesthetic quality materials. Trichloroethylene was purified by fractional distillation. Dibromotrifluoroethane, kindly supplied by Dr C. W. Suckling, had been specially purified. No impurities could be detected when a sample was examined in the mass spectrometer.

Statistical treatment of results

The results for each test on one chemical were in the form of a group of 120 numbers in twenty-four groups of five. The observations were made in four groups centred on times 30, 60, 120 and 240 min. from the beginning, each on five different doses of the chemical with one control containing none of the chemical.

The first step in the treatment of results was to average the groups of five to give twenty-four results, one for each of five different doses plus a control, at each of the four times. When the average figures for grain weevils knocked down were converted to probits and plotted, it was found that there was a roughly linear relationship between the probit of the knock-down, the logarithm of the exposure time, and the logarithm of the thermodynamic activity. We therefore treated the results by the probit-plane method described by Finney (1952), the appropriate correction being applied for the knock-down in the control. Each flask was numbered and controls were arranged so that each flask served at least once as control in each set of seven experiments. A series of blank runs without chemical present showed that the major source of variation was between flasks, and the appropriate correction for control knock-down in any flask therefore was taken to be the blank value obtained in that flask during the current set of experiments.

Calculations by the methods described by Finney resulted in an equation of the regression plane of the form

$$Y = A + b_1 \log \alpha + b_2 \log t$$

and an estimate of the goodness of fit in the form of χ^2. If χ^2 was large (>34) a new set of values for Y was calculated from the equation and the calculation repeated. Usually this resulted in an adequate fit, although in a few cases it has been necessary to repeat once more the fitting process.

The thermodynamic concentration of chemical necessary to produce a 50% effect in 2 hr.—the ED 50 (2 hr.)—was taken as a characteristic figure for each chemical. This value was obtained as the logarithm of a thermodynamic activity by substituting 5·0 for Y and 0·3 for $\log t$ in the equation above and solving for α.

The constant A and the two coefficients b_1, b_2 may be estimated from the approximate planes fitted by eye if it is desired to avoid the mathematical analysis. The slopes of the two sets of parallel lines give b_1 and b_2. The constant A may be estimated by choosing any point on the plane and substituting the appropriate values for Y, b_1, b_2, $\log \alpha$ and $\log t$ in the equation $Y = A + b_1 \log \alpha + b_2 \log t$ and solving for A. In a similar way any characteristic value chosen as a basis for comparison of narcotics may be estimated from the planes.

The values of the ED 50 estimated from the graphical approximations to the probit planes are usually in excellent agreement with the figures resulting from the mathematical treatment of the results, as inspection of Table 1 will show. The estimates of the slopes b_1 and b_2 are much more erratic, and for the best estimates of these the mathematical treatment is essential.

RESULTS

The results of the experiments are summarized in Table 1. For detailed steps in the calculation leading to these results reference must be made to Finney (1952).

Table 1 sets out the results obtained in triplicate for each of the six chemicals. An attempt was made to combine the three separate planes into one for each chemical but the analysis of variance showed the mean square due to departure from parallelism of the three planes to be significant when tested against the mean square of residual heterogeneity. There was no justification for combining to get a single estimate of the parameters, and the results of each experiment have therefore been shown separately. It will be seen that whilst there is wide variation in the values obtained for the constant A and the coefficients b_1 and b_2 the selected characteristic log ED 50 (2 hr.) shows good agreement between the replicate determinations.

The values for log ED 50 (2 hr.) for chloroform and for ether are very close to the thermodynamic concentrations quoted by Ferguson (1951) for the threshold narcosis of grain weevils with these chemicals (chloroform 0·01, ether 0·01, that is, antilog $-2{\cdot}0$ in each case).

The dibromotrifluoroethane $CF_3.CHBr_2$ described by Robbins (1946) is shown in our experiments to be equal to chloroform and ether in narcotic potency to grain weevils. *cyclo*Propane is slightly less potent, whilst vinyl ether and trichloroethylene are the least potent of the six compounds examined.

The standard errors of the coefficients of the probit plane shown in Table 1 have been calculated as described by Finney (1952).

Observation of the mortalities 72 hr. after the conclusion of the narcosis experiment showed that one of the halogenated hydrocarbons, dibromotrifluoroethane, gave 100% kill of grain weevils at the higher dosages. Trichloroethylene produced some kill in one experiment, and the other chemicals were without effect.

TABLE 1. *Summary of results*

	Coefficients of equation $Y=A+b_1 \log \alpha+b_2 \log t$					Log ED 50 (2 hr.)		
Compound	A	b_1	b_2	$n=\frac{b_1}{b_2}$	Fiducial limits (5 %) of n	Calculated (from equation)	Estimated (from probit plane fitted by eye)	χ^2
Chloroform	13·3	4·6±0·64	1·7 ±0·40	2·7	1·8 to 4·9	−1·9	−1·9	6·3
	12·8	4·3±0·56	1·7 ±0·40	2·5	1·7 to 4·5	−2·0	−2·0	8·0
	10·5	2·9±0·50	1·5 ±0·34	1·9	1·2 to 3·5	−2·1	−2·1	24·2
Dibromotrifluoroethane	11·3	3·5±0·48	1·7 ±0·45	2·1	1·3 to 4·4	−2·0	−2·0	2·9
	10·8	3·2±0·41	1·6 ±0·33	2·0	1·4 to 3·3	−2·0	−2·0	7·9
	10·9	3·1±0·51	1·7 ±0·34	1·8	1·2 to 3·0	−2·1	−2·0	4·2
Trichloroethylene	8·8	2·4±0·37	1·7 ±0·32	1·4	0·9 to 2·3	−1·8	−1·8	15·6
	10·6	3·7±0·63	1·8 ±0·39	2·1	1·4 to 3·4	−1·6	−1·7	9·6
	9·8	3·0±0·45	1·7 ±0·36	1·8	1·2 to 3·0	−1·8	−1·9	14·4
Ether	17·7	6·9±0·69	0·001±0·35	6900	<−9·3 or >9·3*	−1·9	−1·8	10·1
	15·8	5·6±0·65	1·5 ±0·38	3·7	2·5 to 7·4	−2·0	−2·1	14·9
	13·8	4·4±0·75	0·61 ±0·46	7·2	<−9·0 or >3·3*	−2·0	−2·1	19·4
*cyclo*Propane	11·5	3·7±0·51	−0·23 ±0·32	−16·1	<−2·9 or >6·5*	−1·8	−1·8	32·7
	13·9	4·8±0·51	−0·04 ±0·33	−120·0	<−6·7 or >6·7*	−1·9	−1·9	11·2
	10·4	3·0±0·36	−0·25 ±0·28	−12·0	<−3·4 or >8·1*	−1·8	−1·9	20·2
Vinyl ether	8·2	1·9±0·48	−0·08 ±0·35	−23·8	<−1·7 or >2·9*	−1·7	−1·7	7·4
	13·1	4·5±0·43	0·08 ±0·30	56·3	<−7·5 or >6·5*	−1·8	−1·9	9·9
	14·1	5·0±0·53	0·21 ±0·31	23·8	<−10·2 or >5·6*	−1·8	−1·8	5·5

* Where the value of the denominator, b_2, is not significantly greater than zero the fiducial range for n consists of all values *not* lying between the roots of Fieller's equation (Fieller, 1944).

DISCUSSION

The method of measuring narcosis described in this paper uses an easily bred organism which can be treated in sufficient numbers to permit reproducible values for narcotic potencies. A significant departure has been made from previous experimental methods for the narcosis of grain weevils by the establishment of a quantitative measure of the degree of narcosis.

The application of Finney's probit-plane treatment to the numbers of insects affected gives a simple picture of the reaction of the organism to the chemical under test and, in the chemical anaesthetics examined, there is a consistently good fit of the experimental points (as demonstrated by the χ^2 test) to the probit plane described by the equation

$$Y=A+b_1 \log \alpha+b_2 \log t.$$

A rough approximation to the probit plane can often be obtained without lengthy calculations by plotting the probit of percentage insect knock-down (appropriately adjusted for control knock-down) against log thermodynamic concentration plus log time and drawing through these points the plane estimated by eye to give the best fit, subject to the restriction that the lines of the plane shall be parallel.

A better fit of the plane to the points results from application of the maximum likelihood method of calculation described by Finney (1952) to the working probits read off from the estimated plane; this is rather time-consuming and may not be necessary if the method is being used to determine a characteristic of potency such as the ED 50 (2 hr.). If the coefficients b_1 and b_2 are important then the mathematical treatment must be applied. It is also essential, of course, if a reasonably good fit to the points cannot be made by eye.

The equation of the probit plane gives a concise description of the behaviour of the chemical under test. The constant A is the value assumed by Y when $\log \alpha$ and $\log t$ are both zero, that is, A is the probit of the narcotic effect of a saturated vapour after 1 hr. exposure. It may be taken as an indispensable feature of effective anaesthetic potency that this value should not be less than 8·0, the probit of 99·9% knock-down.

The magnitudes of the coefficients b_1 and b_2 are descriptive of the behaviour of the insects in the atmosphere of narcotic vapour. If b_1, the regression of response on thermodynamic concentration, is large the insects respond markedly to changes in thermodynamic concentration of vapour in the surrounding atmosphere. If a connexion between probit of knock-down and depth of anaesthesia may be assumed, a large value of b_1 might be considered a valuable attribute in that it might permit sensitive control of anaesthesia by regulation of the partial pressure of the chemical being applied.

The regression of response on log time, b_2, describes the behaviour of the insects when exposed for a period to a fixed thermodynamic concentration of narcotic. If b_2 differs significantly from zero it shows that the dose required to produce any given response may be a function of time as well as of thermodynamic concentration. A high value of b_2 means that the biological effect of a given concentration of vapour increases sharply so long as the insects are held in it, whereas a low value indicates that the degree of response is not greatly affected by the length of exposure. In the case of three of the narcotics examined the effect of time is slight, b_2 being close to zero. It must be remembered that the value we have measured is the coefficient of a logarithmic expression of time and is based on measurements during the period $\frac{1}{2}$–4 hr. after application of the vapour. Thus low values of b_2 for *cyclo*propane or ether do not mean that these did not cause a rapid initial response of the insects exposed to them. Inspection of the probit planes for *cyclo*propane, ether and vinyl ether showed that when the first measurements were taken $\frac{1}{2}$ hr. after the start of exposure almost the full response had been produced and that thereafter there was little change. The figure b_2 cannot be said to have any clear relation to the 'rate of induction' (Nisbet, 1949) in clinical anaesthesia, therefore, but it may be indicative of the nature of the response. A very small coefficient of log time of exposure is indicative of a steady state which might be the result of physical equilibrium between the narcotic in the atmosphere of the flask and the site of action in the grain weevil. Some of the anaesthetics thus approximate closely to the requirements for Ferguson's principle of thermodynamic equilibria—a rapid arrival at a state of reversible equilibrium.

A large value of b_2, on the other hand, is indicative of a progressive change in the biological effect of an applied thermodynamic concentration and is compatible with a state of reversible equilibrium only if a gradually increasing biological response to a fixed level of concentration of chemical at the site of action be postulated.

It has been pointed out (Healy, 1953) that the probit-plane technique is not wholly appropriate to the results reported here, since successive readings are made on the same batch of insects and are therefore not independent. Because of this, not much reliance can be placed on the standard deviations of the estimates of b_2 recorded in Table 1. It is clear, however, that the six compounds examined fall into two groups with regard to the values of b_2. Vinyl ether, *cyclo*propane, and ether have low values, whereas chloroform, trichloroethylene, and dibromotrifluoroethane have larger positive values of b_2. Unless it be assumed that the three halogenated compounds are slow in penetrating to the site of action (which seems unlikely in view of the marked response to differences in concentration even after half an hour) the positive value of b_2 must mean that the effect of a given thermodynamic concentration of these compounds in the affected phase of the biological system progressively increases.

If the drug in the affected phase undergoes no chemical reaction the increasing interference with the normal behaviour of the insects must be seen as the mounting response to a constant physical stimulus. This can be compared with the 'physical toxicity' which Ferguson & Pirie (1948) describe as toxicity which is due to a chemical in the affected phase interfering with the normal behaviour of the insects without undergoing chemical reaction.

On the other hand, a positive value of b_2 significantly greater than zero might be interpreted as indicative of a slow chemical reaction following the access of the drug to the site of action.

Biological actions which follow the probit-plane pattern

$$Y = A + b_1 \log \alpha + b_2 \log t$$

can also be expressed as $\lambda^{b_1} t^{b_2} = \text{constant}$ for any selected value of the probit Y, λ and t being the absolute (not logarithmic) measures of dose and time. In the form $\lambda^{b_1/b_2} t = \text{constant}$ the equation becomes $\lambda^n t = k$ (Finney, 1952; Busvine, 1938).

The six anaesthetic compounds examined in our work all have b_1, the coefficient of log thermodynamic concentration, greater than b_2 the coefficient of log time, so that the value of $n = b_1/b_2$ is greater than unity (see Table 1). Clark (1937*b*) reports the results of Knoefel and Murrell with ether for the production of anaesthesia in mice, when $C^{2\cdot 5} t = k$.

Chemicals producing irreversible toxic action frequently show a value of n close to unity. Thus hydrogen cyanide in its toxic action on grain weevils approximates to the formula $Ct = k$ (Finney (1952); calculations based on results of Peters and Ganters). More recently, Lanchester (1951) has found the action of DDT on wireworms to follow a similar relationship, the points on the probit/log concentration + log time graph being fitted by a single straight line.

As b_2 approaches zero n increases and for reversible physical action a large value of n may be expected. The value of n may become infinite with chemicals which show no change in biological action with time of exposure. From the evidence quoted above, it appears that irreversible toxic action may be indicated by a value of n approaching unity, which is associated with increasing b_2, the coefficient of log time of exposure to the drug.

It is interesting in this connexion to note that Busvine (1938) concluded that the value n must be mentioned in expressing resistance of insects to the toxic action of vapours, larger values of n indicating relatively greater resistance of insects to prolonged exposures.

It must be made clear, however, that as a ratio of two variables b_1 and b_2, estimates of n may be expected to vary over a wide range. The fiducial limits of n have been calculated by the equation of Fieller (1944), using the estimated variances and covariance of b_1 and b_2 resulting from the probit-plane analysis. These are shown in Table 1. The wide ranges covered by the fiducial limits for n calculated from our own experiments suggest a reason for the very great variation in the values taken by n in the expression $C^n t = \text{constant}$ fitted by various workers in different laboratories to experimental results on one and the same compound. Many examples of the extent of this variation are to be found in Clark (1933).

The six compounds tested in our experiments fall into two groups. Vinyl ether, *cyclo*propane and ether have high values of n and can be said to show reversible physical effects on the grain weevil. The three halogenated hydrocarbons have lower values of n and may have some irreversible toxic action on the grain weevil. This is supported in the case of dibromotrifluoroethane by some kill of the weevils at the higher thermodynamic concentrations.

The authors are indebted to Dr J. Ferguson for suggesting the topic and for valuable discussions and criticism. They also express their thanks to Dr A. Campbell for his collaboration in the later stages of the work, and to Miss H. M. S. Midwood and Mr W. R. Curry for assistance with the experimental work and calculations.

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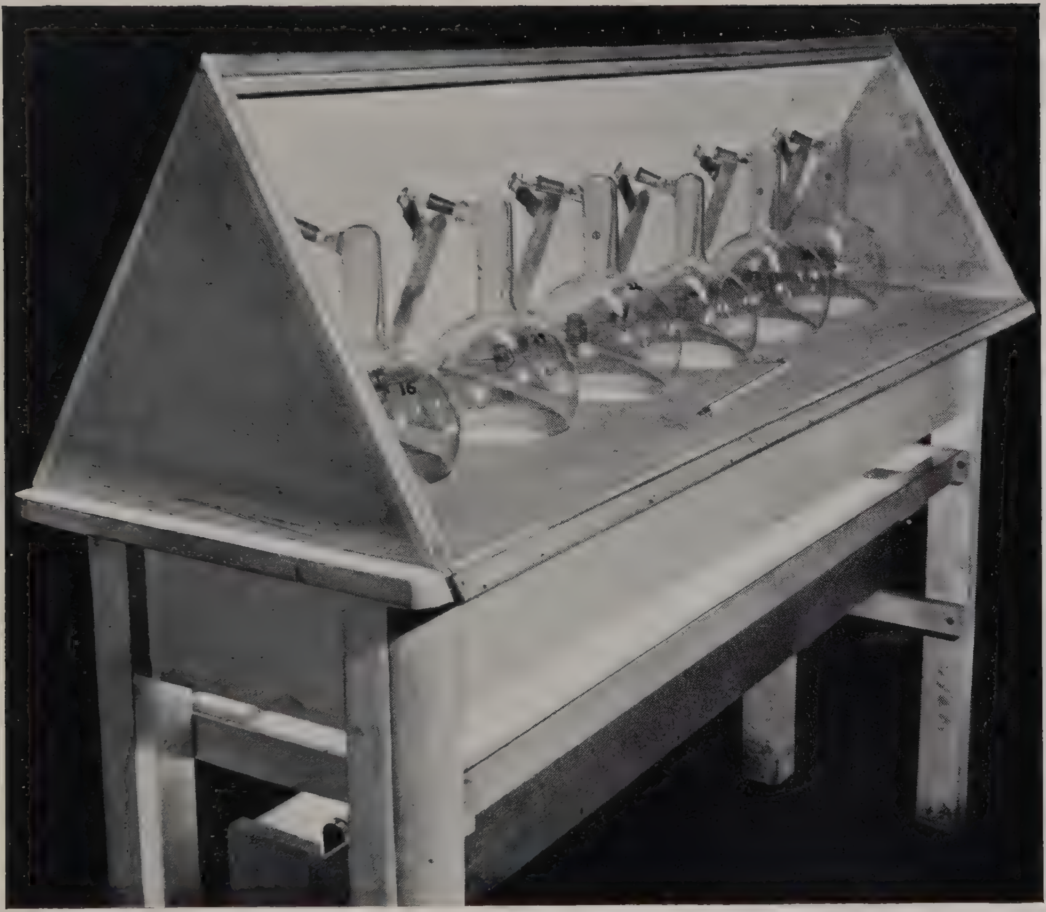
EXPLANATION OF PLATE 7

Apparatus for exposing insects to narcotic vapours.

(*Received* 12 *February* 1953)



BRADBURY & ARMSTRONG—*Measuring the narcotic action of chemicals*

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THE EFFECT OF BODY WEIGHT ON THE RESISTANCE TO INSECTICIDES OF THE LAST-INSTAR LARVA OF *DIATARAXIA OLERACEA* L., THE TOMATO MOTH

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(With 6 Text-figures)

At a constant temperature of 24° C. the final larval instar of *Diataraxia oleracea* lasts about 10 days, during which its resistance to DDT and γ-BHC as contact insecticides progressively increases up to the 5th or 6th day. It then suddenly decreases, this coinciding with cessation of feeding and the beginning of prepupal formation.

Between the 2nd and the 6th days the gross body weight of the last-instar larva increases from about 0·27 to 0·65 g. Under the conditions of the experiments, the LD 50 of parathion, as a stomach poison, was linearly related to body weight; on the same basis TEPP was slightly less, and lead arsenate, slightly more, toxic to the larger than to the smaller larvae. However, DDT as a stomach or contact insecticide, and γ-BHC as a stomach poison were notably less toxic to the larger larvae. For example, the increase in LD 50 for an increase in larval body weight of × 2 was about × 11 for DDT as a stomach poison and about × 12 as a contact insecticide.

The order of effectiveness of the above insecticides as stomach poisons for the last-instar larva of *D. oleracea* was parathion $>$ DDT $>$ γ-BHC $>$ TEPP $=$ lead arsenate. Zinc fluoarsenate and rotenone were relatively non-toxic. Larvae of *D. oleracea* were repelled by food leaf treated with an extract of natural pyrethrins.

INTRODUCTION

Animals used in pharmacological and toxicological work are often of different sizes, and it is standard practice to express dose as a ratio of gross body weight. Thus the dose of a stomach insecticide is given in milligrams of insecticide per gram of insect body weight. This procedure is based on the assumption that the physiological response of an organism is directly proportional to the body weight, an assumption which has been questioned by Moore (1909), who suggested that sometimes susceptibility was more likely to be proportional to the surface of absorptive tissues than to the body weight direct. Durham, Gaddum & Marchal (1929) also appreciated that dosage is not always directly proportional to body weight, while Bliss (1936), using data of Campbell's (1926*a*, *b*) on the toxicity of arsenic to silkworm larvae, showed that, using survival time to measure toxicity, the larger larvae died more slowly than did smaller larvae which had been given the same amount of arsenic per gram of body weight. Bliss proposed a 'size factor' which he defined as a function of size (in this case body weight), determining the amount of poison to be given to each individual to produce equivalent toxic action (in this case survival time).

Percentage mortality is generally used as the criterion of insecticidal toxicity, and for different insects and insecticides the median lethal dose is often given as a ratio of gross body weight, although there is no evidence that this assumption is valid.

This paper describes work on the relationship between body weight of the last-instar *D. oleracea* larvae and its resistance to several insecticides.

MATERIALS

Larvae of *D. oleracea* were reared at a constant temperature of 24° C. as described by Way, Smith & Hopkins (1951); those in the last instar were selected for the tests.

The following insecticides were used:

Acid lead arsenate, 98–99% pure; mean diameter of particles about 5 μ.

Pure 2:2-bis-(*p*-chlorophenyl)-1:1:1-trichloroethane (DDT).

Pure gamma-hexachlorocyclohexane (γ-BHC).

Commercial tetraethyl pyrophosphate (TEPP) containing about 40% pure material.

Commercial *O*:*O*-diethyl-*O*-para-nitrophenylthiophosphate (parathion) containing about 80% pure material.

The doses of TEPP and parathion are given in terms of the commercial product as the exact content of toxicant was not known nor was it known whether the impurity consisted of insecticidal material.

Certain insecticides proved unsuitable owing to their low toxicity or repellent effect. Thus, in preliminary experiments zinc fluoarsenate was not toxic even at a dose of 0·37 mg. per larva, and thiodiphenylamine, non-toxic at 0·19 mg. per larva. Pure rotenone affected the larvae but did not kill at 0·20 mg. per larva. The LD 50 of chlormethyl chlorphenyl sulphone was about 0·4 mg. per larva for larvae weighing 0·35–0·45 g. but those weighing more than 0·5 g. were not killed by a dose of 0·49 mg. The larvae were violently repelled immediately they bit a leaf area treated with an extract of natural pyrethrins.

METHODS

Last-instar *D. oleracea* larvae were fed with stomach poisons as described by Way (1949). Each was given a 48 sq.mm. area of cabbage leaf bearing a known deposit of insecticide obtained by treating leaves in a settling mist tower. Inorganic insecticides were sprayed as a suspension in water, and organic insecticides as a solution in acetone. The settling mist tower was not used for TEPP, which could decompose during the 1–2 hr. period between spraying the leaves and feeding them to the test insects. Accordingly, for this insecticide unsprayed leaf areas were fitted in feeding apparatus (Way, 1949) and with a micropipette were treated with 0·001 c.c. of ethyl alcohol containing the required dose of TEPP. Two to three minutes were allowed for the alcohol to evaporate before the leaf areas were given to the test insects. In this way decomposition of TEPP, before its consumption, was avoided.

For the experiments, each larva was weighed and treated separately; only about 150 could be dealt with each day and consequently some experiments lasted 2 or 3 days. For each day's work about 150 last-instar larvae of varying size were divided at random into four or five equal batches, and the larvae weighed individually. Each batch of larvae was then fed with one of four to five doses of insecticide.

The Potter spray tower (Potter, 1941) was used for work on contact toxicity. The doses of contact insecticide retained by larvae after spray treatment with DDT were estimated by stripping the insecticide deposits from the larval cuticle immediately after spraying and analysing them, using the method of Schechter, Soloway, Hayes & Haller (1945).

During and after treatment the larvae were kept individually in cages at a constant temperature of 24° C. After treatment they were given fresh food. A larva was considered to have recovered if it pupated normally or if it began to feed and to increase in weight.

Statistical analysis of the toxicity data was carried out using the method of probit planes (Finney, 1943) as described by Way (1949).

GROWTH OF THE LAST-INSTAR *DIATARAXIA OLERACEA* LARVA

Fig. 1 shows the rate of development of the last-instar *D. oleracea* larva reared at a constant temperature of 24° C. The larva feeds during the first 5–6 days and the weight increases from about 0·15–0·2 to 0·6–0·7 g. At about the 6th day the larva abruptly ceases feeding, evacuates the gut, seeks for a suitable site for pupation and by the 8th day has built a cocoon and become a prepupa. The weight loss between the 5th and 8th days is caused mainly by evacuation of the gut and by transpiration. Pupation occurs between the 9th and 10th day.

Stomach insecticides can be administered only during the feeding stage of the larva when age (within the instar) and body weight are positively correlated (Fig. 1).

EFFECT OF BODY WEIGHT ON TOXICITY OF STOMACH POISONS

The results of experiments with lead arsenate, parathion, and DDT are shown graphically in Figs. 2–4. Each dot or ring denotes a single larva, and the curve is the calculated one showing the relationship between the LD 50 (in mg. insecticide per larva) and the larval body weight (g.).

Table 1 shows the summarized results in which, for analysis, each batch of larvae was subdivided into three weight groups with average weights of 0·32, 0·42 and 0·56 g. respectively. In experiments using DDT, some larvae did not consume the whole dose presented to them, and consequently the amount eaten was calculated from the area of leaf consumed. Fig. 4 shows that this has caused a scatter of some of the points, and for analysis it was necessary to make dosage groups as well as body weight groups.

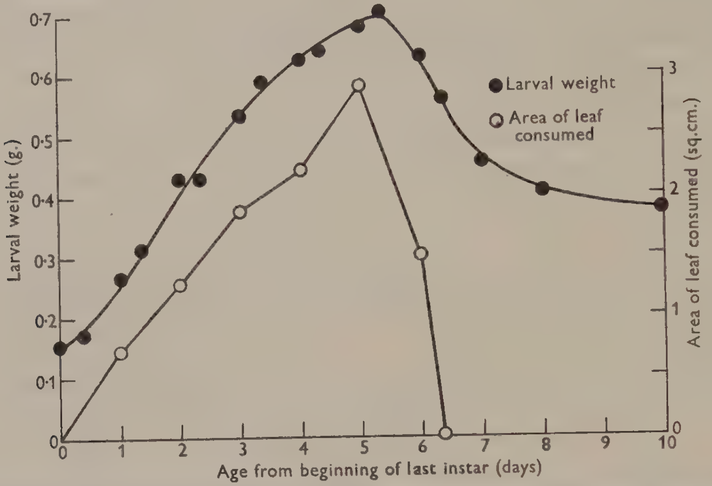

Fig. 1. The change in body weight during the last instar of a single *D. oleracea* larva fed on cabbage foliage and reared at a constant temperature of 24° C.

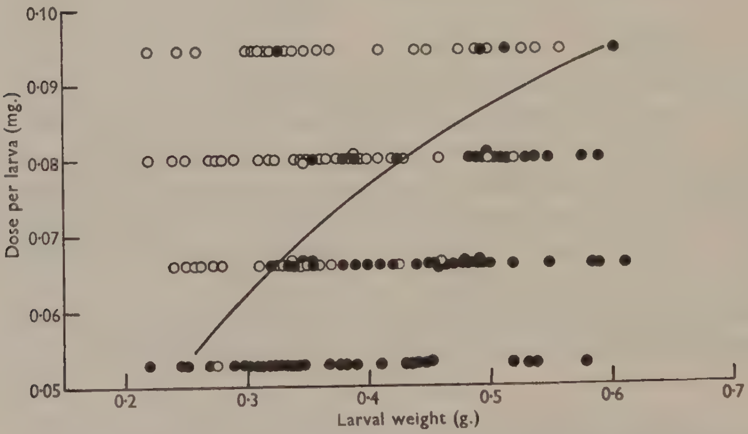

Fig. 2. The effect of acid lead arsenate as a stomach poison for last-instar larvae of *D. oleracea* of varying body weights. ○, insects which died; ●, insects which recovered or were unaffected.

Table 2 shows the results of analysis using probit planes of the form

$$Y = a + b_1 x_1 + b_2 x_2,$$

where x_1 = log weight (g.) + 1 and x_2 = log dose (mg.) + 4.

Table 3 gives the LD 50's in mg. insecticide per larva for each weight group and also the ratios of LD 50's for larvae with a body weight ratio of 1:2. It can be seen that the size factors differ according to the insecticide. The relatively high resistance of larger larvae to DDT and γ-BHC is noteworthy; clearly resistance of *D. oleracea* to these insecticides is not a function of gross body weight.

Parathion is extremely poisonous to the *D. oleracea* larva, and if the pure

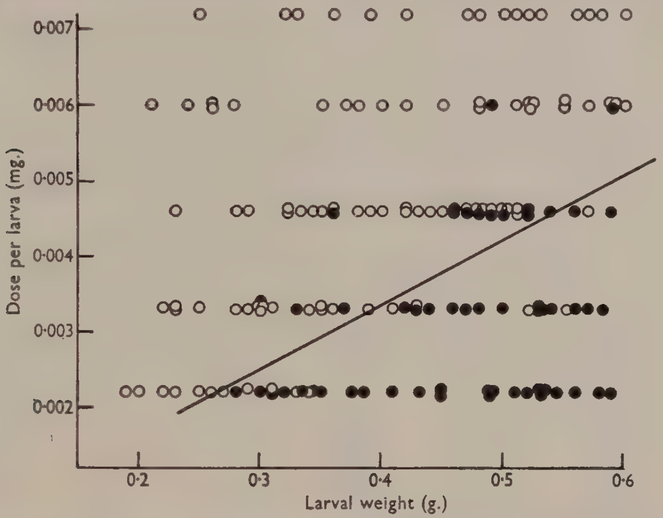

Fig. 3. The effect of parathion as a stomach poison for last-instar larvae of *D. oleracea* of varying body weights. ○, insects which died; ●, insects which recovered or were unaffected.

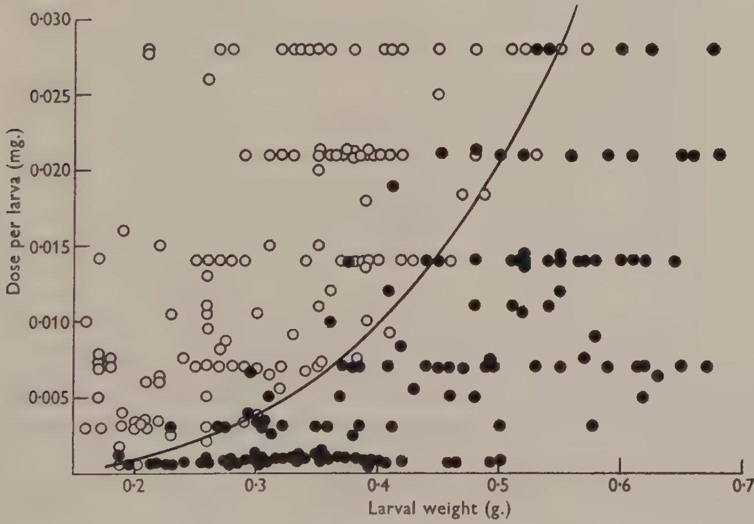

Fig. 4. The effect of DDT as a stomach poison for last-instar larvae of *D. oleracea* of varying body weights. ○, insects which died; ●, insects which recovered or were unaffected.

material is the only toxic constituent of the commercial product used in this experiment, the true LD 50's are approximately four-fifths of those given in the table. However, it is notable that on the basis of the LD 50, parathion is only 1·7 times more effective than DDT against smaller larvae (0·32 g.) but 7·2 times more effective against the larger (0·56 g.). Based on the LD 50's the order of toxicity of the various insecticides was parathion > DDT > γ-BHC > TEPP = lead arsenate.

TABLE 1. *Toxicity of insecticides as stomach poisons to last instar-larvae of* Diataraxia oleracea *showing the percentage kills of larvae of three different weight groups caused by a range of dosages of insecticides*

		Mean larval weights (g.)					
		0·32		0·42		0·56	
	Dose/larva (mg.)	No. of insects	Percentage kill	No. of insects	Percentage kill	No. of insects	Percentage kill
Lead arsenate	0·053	13	8	10	0	4	0
	0·067	14	57	18	17	9	0
	0·080	12	92	13	69	13	15
	0·094	10	90	5	100	8	50
TEPP	0·015	10	0	9	0	6	0
	0·032	15	40	9	0	5	0
	0·064	13	77	14	14	9	11
	0·096	9	100	16	81	15	40
	0·131	6	100	12	100	19	74
Parathion	0·0022	12	42	6	0	12	0
	0·0033	12	83	11	36	10	20
	0·0046	9	89	15	73	13	46
	0·0060	2	100	7	100	12	83
	0·0072	3	100	4	100	8	100
γ-BHC	0·007	14	0	10	0	2	0
	0·013	13	54	6	17	7	0
	0·024	19	90	33	52	13	8
	0·038	11	100	13	92	17	6
	0·051	10	100	13	85	14	36
	0·064	6	100	16	94	15	53
DDT	0·00075	22	0	10	0	2	0
	0·0030	13	15	6	0	3	0
	0·0070	13	85	13	31	12	0
	0·014	9	100	15	60	19	0
	0·021	10	100	18	83	7	14
	0·028	7	100	7	100	8	50

TABLE 2. *Probit analysis results of experiments to determine the effect of body weight on susceptibility of last-instar* Diataraxia oleracea *larvae to various stomach poisons*

Insecticide	a	b_1	b_2	x_2
Lead arsenate	−32·3	− 8·6	+14·8	1·44 (2 D.F.)
TEPP	− 5·1	− 9·6	+ 5·6	5·82 (3 D.F.)
Parathion	− 1·7	− 7·3	+ 7·3	1·65 (3 D.F.)
γ-BHC	+ 1·3	−12·9	+ 4·8	5·95 (4 D.F.)
DDT	+ 5·5	−16·2	+ 4·6	2·55 (2 D.F.)

EFFECT OF BODY WEIGHT ON CONTACT TOXICITY

All the insecticides except lead arsenate cause regurgitation some time after being consumed by the *D. oleracea* larva, and thus some of the dose may be lost. Such loss would not occur if the chemical was applied as a contact insecticide.* This was studied using DDT.

The dose of a contact insecticide is normally expressed as the concentration of toxic material in the spray or dust with which the insect is treated, the amount of insecticide adhering to each insect being unknown. It would partly depend on the surface area of the cuticle and thus larger larvae of *D. oleracea* would receive a heavier dose relative to concentration than the smaller larvae.

TABLE 3. *Values of the LD50 in mg. per larva of various insecticides as stomach poisons for last-instar larvae of* Diataraxia oleracea *of three different body weights*

	Larval weight (g.)			Ratio of LD50 for body weight ratio 1:2
Insecticide	0·32	0·42	0·56	
Lead arsenate	0·066	0·078	0·091	1:1·5
TEPP	0·043	0·069	0·112	1:3·3
Parathion	0·0026	0·0034	0·0046	1:2·0
γ-BHC	0·013	0·026	0·059	1:6·5
DDT	0·0045	0·012	0·033	1:11·2

Known doses of contact insecticide can be applied as droplets using a micropipette; however, the toxic effect varies according to the area of cuticle covered by the droplet (Way, unpublished) which presumably should be proportional to the surface area of the insect. This method proved impracticable for the soft-bodied *D. oleracea* larva, and the one used was to spray batches of five to ten larvae in 9 cm. Petri dishes (Whatman no. 1 filter-paper base) using the Potter tower (Potter, 1941). Treated larvae were kept in the Petri dish for exactly 5 min. to allow excess insecticide to drain from the cuticle and were then transferred to separate cages and kept with fresh food at a constant temperature of 24° C. In this way five batches of larvae, each containing individuals of varying size, were sprayed with a range of five concentrations of DDT emulsion in a medium containing 5% (v/v) benzene as solvent and 0·05% (w/v) cyclohexylamine dodecyl sulphate as emulsifier. Kills were recorded as previously described.

The doses of insecticide retained by the larval cuticle were determined by treating larvae as above with known concentrations of DDT spray and then stripping the DDT deposits from the larval cuticle and analysing them chemically as described by McIntosh (1947). The stripping procedure was carried out exactly 10 min. after treatment, by which time excess spray had run off and the spray deposit remaining on the larval cuticle had dried.

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* Insecticides such as parathion, DDT and γ-BHC cause regurgitation both when injected into the body cavity and when applied as contact insecticides. Thus regurgitation is not necessarily due to irritation of the gut.

Table 4 shows that, as expected, the dose retained by larvae of the same weight range is proportional to the concentration sprayed.

The weights of DDT retained by larvae of different body weight sprayed with a fixed concentration of DDT are shown graphically in Fig. 5. Thirty to forty-five larvae were used to determine each point. Thus, knowing the larval weights and the concentrations of DDT spray used in the toxicity studies, the doses of insecticide retained by each larva could be calculated. The calculated doses and the percentage kills of five weight groups of last-instar *D. oleracea* larvae treated with a range of concentrations of DDT emulsion are given in Table 5.

TABLE 4. *Weights of insecticide retained by the cuticle of larvae sprayed with 0·1% (w/v) and 1·0% (w/v) DDT emulsion*

Larval weight (g.)				
Wt. range	Mean wt.	No. of insects	Percentage (w/v) DDT in spray	Dose DDT retained/larva (mg.)*
0·16–0·65	0·30	80	0·1	0·0057
		80	1·0	0·0541

* The atomizing nozzle used for spraying in this experiment differed from that used in later experiments. It may be responsible for the relatively high deposits of DDT retained by the larvae.

TABLE 5. *The percentage kills of various weight groups of last-instar* Diataraxia oleracea *larvae treated with a range of doses of DDT as a contact insecticide*

Mean larval wt. (g.) ...	0·24			0·34			0·44		
DDT (w/v) in spray	Dose mg./larva	No. of larvae	% kill	Dose mg./larva	No. of larvae	% kill	Dose mg./larva	No. of larvae	% kill
0·015	0·00041	27	30	0·00061	19	11	0·00081	8	0
0·033	0·00089	11	82	0·0014	19	53	0·0018	3	33
0·068	0·0018	4	100	0·0028	12	83	0·0037	18	28
0·141	0·0038	8	100	0·0058	6	100	0·0076	19	100
0·295	—	—	—	0·0121	3	100	0·0159	3	100

Mean larval wt. (g.) ...	0·54			0·71		
DDT (w/v) in spray	Dose mg./larva	No. of larvae	% kill	Dose mg./larva	No. of larvae	% kill
0·015	0·00096	8	0	0·0011	6	0
0·033	0·0021	8	0	0·0024	7	0
0·068	0·0044	8	0	0·0049	6	0
0·141	0·0090	25	40	0·0102	5	0
0·295	0·0189	23	100	0·0212	6	33

TABLE 6. *Values of the LD50 of DDT as a contact insecticide for last-instar larvae of* Diataraxia oleracea *of different body weights*

Larval wt. (g.)	0·24	0·34	0·44	0·54	0·71
LD50 (mg./larva)	0·00047	0·0016	0·0041	0·0087	0·024

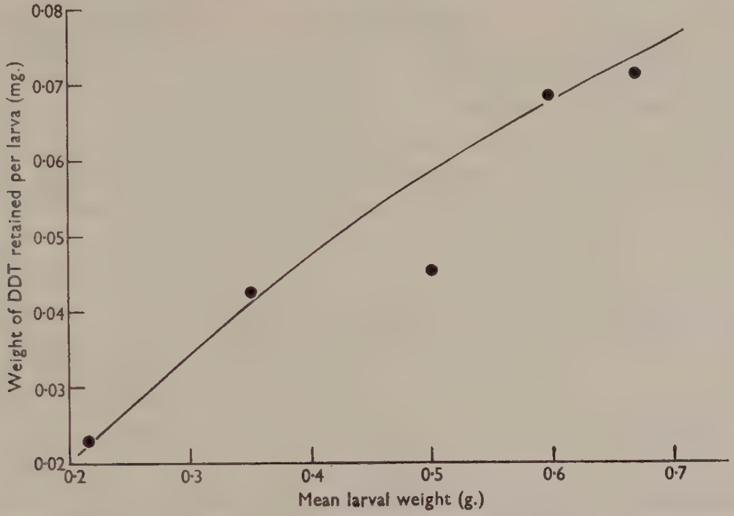

Fig. 5. The weights of DDT retained by last-instar larvae of *D. oleracea* of varying body weights sprayed with an emulsion containing 1 % (w/v) DDT.

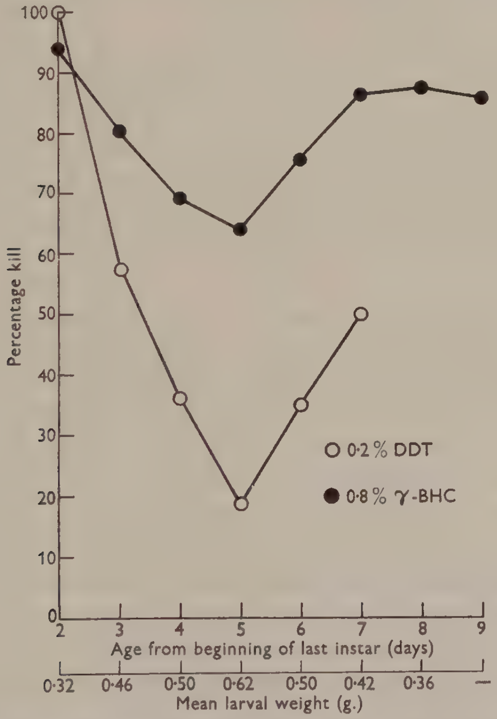

Fig. 6. The percentage kills in batches of last-instar larvae of *D. oleracea* of different ages after treatment with 0·2 % (w/v) DDT and 0·8 % (w/v) γ-BHC as contact insecticides.

The results of probit plane analysis in which x_1 = log weight (g.) + 1 and x_2 = log dose (mg.) + 4 were: $a = +8\cdot3$; $b_1 = -16\cdot9$; $b_2 = +4\cdot7$; $\chi^2 = 5\cdot52$ (3 D.F.). The values of the LD 50's are given in Table 6.

The results show that for a larval body weight ratio of 1:2, the ratio of LD 50's of DDT as a contact insecticide was 1:12·2 compared with 1:11·2 for DDT as a stomach poison. It is noteworthy that in these experiments DDT as a contact insecticide was approximately 3 times more toxic than as a stomach poison.

SUSCEPTIBILITY OF LATE LAST-INSTAR LARVAE

The foregoing experiments were carried out using larvae in the feeding stage, but some data on contact toxicity of DDT and γ-BHC to older larvae were also obtained; the larvae were reared individually at 24° C. from the beginning of the last instar and thus were of known age. Fig. 6 shows the kills in batches of about thirty larvae after treatment with 0·2% (w/v) DDT and 0·8% (w/v) γ-BHC in the benzene, *cyclo*hexylamine dodecyl sulphate emulsion. As before, resistance increased during the feeding stage, but after the 5th day, when feeding ceases, there was a sudden decrease in resistance. Although this coincided with a decrease in body weight, it also coincided with a marked change in physiological state from the feeding to the moulting phases of larval development.

DISCUSSION

The physiological response of an organism to a certain dose of drug depends on the amount of the body constituent directly affected by the drug, and also on many other factors, including the permeability of the gut or body wall, and various processes which inactivate or excrete the drug. Therefore, when dose is expressed as a ratio of gross body weight, it is being assumed that the combined activity of the many factors which determine the organism's response to a drug is a function of the body weight.

The work described in this paper has shown that, based on LD 50's, the resistance of the last-instar *D. oleracea* larva to parathion, TEPP and lead arsenate is closely correlated with the larval body weight. Therefore, it seems that for these insecticides the above assumption is valid. However, relative to body weight, the larger *D. oleracea* larvae are more resistant than smaller larvae to γ-BHC as a stomach poison, and much more resistant to DDT as a contact or stomach poison; with the latter a doubling of larval body weight causes an increase in resistance of × 11–12. Therefore the body-weight factor has a notably greater effect on resistance than the dose factor; hence, expressing dose as a ratio of body weight does little to correct the large errors that would occur if the test subjects varied in size. Furthermore, it is evident that for control of lepidopterous larvae in the field DDT and γ-BHC may prove relatively ineffective if used at a late stage of infestation.

I wish to thank Mr M. J. R. Healy for doing the statistical analyses and Mrs J. M. Taylor for much valuable assistance.

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HOST ALTERNATION IN *APHIS FABAE* SCOP.

II. CHANGES IN THE APHIDS

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(With 2 Text-figures)

Experiments on the feeding preferences and relative fecundity of *Aphis fabae* among leaves of different ages and kinds were extended to the comparison of different forms of the aphid: apterous and alate virginoparae and gynoparae.

All three forms showed a preference for the primary host, *Euonymus*, over a secondary host, sugar beet, but this preference was strongest in the gynoparae, weaker in the alate virginoparae and weakest in the apterae. The relative fecundity of the aphids on the two kinds of leaf paralleled their feeding preferences most closely in the gynoparae, less so in the alate virginoparae and least in the apterae.

All three forms also showed some preference for growing over mature leaves of the same kind, but this preference was strongest in the apterous virginoparae, weaker in the alate virginoparae and weakest in the gynoparae. The relative fecundity of the aphids on the two ages of leaf paralleled their feeding preferences most closely in the apterae, less so in the alate virginoparae and least in the gynoparae.

The physiological, ecological and evolutionary significance of the results is discussed in the light of the dual discrimination theory of aphid host selection.

Introduction

Mordvilko (1907–9, 1928, 1936), followed by Davidson (1921, 1927*a*), suggested that the habit of seasonal alternation by many Aphididae between botanically unrelated plants ('heterœcy') in temperate regions gives the aphids more continuous access to nutritionally favourable foliage than they have on any one kind of plant. In support of this it has been shown from observations, and experimentally, that growing and senescing leaves are more suitable for the summer forms of *Aphis fabae* Scopoli than middle-aged, static leaves (Kennedy, Ibbotson & Booth, 1950; Ibbotson & Kennedy, 1950; Kennedy & Booth, 1951). This, and not an intrinsic preference for secondary host plants on the part of these aphid forms, explains their habitual failure to colonize the winter host in the summer. Thus the fact that the woody winter and herbaceous summer hosts are growing or senescing at roughly alternating seasons, was put forward as the probable basis for the evolution of host alternation by the aphids.

Bonnemaison (1951) has since criticized Mordvilko's nutritional theory of host alternation on the grounds that immediate nutrition is not the main determinant for the development of winged migrant forms, either on the primary hosts in spring or on the secondary hosts in the autumn, in *Myzus persicae* (Sulzer) and *Sappaphis mali* Ferrari (=*plantaginea* auct. nec Passerini). But we are concerned here with the past rather than the present causes of host alternation. For the evolutionary causes there is now of course only indirect evidence, based not on the present causes but on the present consequences of host alternation. One consequence, which is often taken for granted, is escape from extinction by natural enemies; another, which has been demonstrated, is a nutritional gain. It remains to be demonstrated that migration is a means of escape in the literal sense of movement to a place where enemies are fewer. But the nutritional gain from movement to new plants will in itself be a means of escape from extinction by enemies, even if they are as numerous in the new place as in the old, because the aphids will multiply faster in the new place. Given that the nutritional gain was what started the evolution of the habit of host alternation, then we might expect, as part of the same evolutionary process, that physiological responses would have developed to associated but non-nutritional 'token' stimuli, or 'conditioning stimuli' to use Pavlov's more objective term. Wing development, for instance, would tend to become a response not only to deficient immediate nutrition, but also to any other stimuli, internal or external, which helped to ensure timely migration to nutritionally more favourable plants. The findings of Bonnemaison and others do not therefore conflict with Mordvilko's main thesis: that the evolutionary origin and the continuing ecological significance of host alternation lie in the changing suitability of the host plants.

The evolution of host alternation on a primarily nutritional basis might be expected to have entrained, in addition, some divergence between the *behavioural* responses of the different seasonal forms of one aphid species. Rich food supplies are available, transiently, to the aphids that can find them, and are exploited by rapid 'vegetative' multiplication of the viviparous forms of spring and summer. Crossing, and passage through the unfavourable season are effected by the sexual forms, the males and egg-laying females. The ecological requirements of the various forms, including their host requirements, are therefore different. Some differentiation of their host preferences would be a natural consequence. Thus Davidson (1927*a*) found that the gynoparae of *Aphis fabae* ('*rumicis*') could be induced to colonize beans only with difficulty. Their sexual offspring matured on the beans but tended to leave them before laying eggs. The experiments now reported were therefore designed to establish how far different forms of *A. fabae* do differ in their preferences and relative fecundity among different kinds and ages of leaf.

MATERIAL AND METHODS

The majority of the virginoparous aphids used were reared on *Vicia faba* on the 'production line' system described by Kennedy & Booth (1950). Early in 1950 this was transferred from the greenhouse to a windowless constant-temperature room, enlarged and simplified; this opportunity may be taken to describe its altered form. The whole breeding operation, including the growing of the bean clumps from seed before they are inoculated with aphids, is carried out within the one room, which is maintained with the help of an air-cooling system at about 10° C. and 75% R.H. Six bean clumps (instead of the previous single one) are inoculated with 100 apterae each on 5 days in the week. The clumps are left unenclosed on a bench illuminated by three 80W. 'daylight' fluorescent lamps giving about 2000 mc. at plant level and running for 16 hr. daily. When the next generation adults begin to wander, the clumps are transferred to a subdivided, closed metal cupboard through which room air is drawn gently but continually by a 'Ventaxia' fan. Here the clumps receive about 500 mc. from fluorescent lamps above the muslin roof of the cupboard, on which the wandering adults gather and can be collected easily with the aspirator 'gun' (Kennedy & Booth, 1950). The temperature is kept low to compensate to some extent for the reduced lighting, but the plants still become drawn and must be supported by a wire-netting frame. The system gives a much larger and more regular output of aphids than when it was in the greenhouse, owing to the greater number and regular growth of the bean clumps and the constant conditions, and yet it takes up no more space.

Some experiments were also done on the virginoparous spring forms of *A. fabae*, apterous and alate fundatrigeniae. These were taken from natural infestations on *Euonymus europaeus* L. (spindle) trees in the garden of the Entomological Field Station, Cambridge, in May 1948. The collections were examined alive under light CO_2 anaesthesia and any specimens of *Aphis euonymi* (Börner & Janisch), or of doubtful affinity, were discarded on the criteria given in Kennedy *et al.* (1950) before caging the aphids for experiment.

Natural infestations on spindle trees in the garden also provided the gynoparae for experiments in September–October, 1948. These infestations were described by Kennedy *et al.* (1950) and did not appear to include any *A. euonymi*. Males were easily distinguished under a hand-lens or even with the naked eye and discarded.

In addition, gynopara-like aphids were produced artificially for one series of experiments in January–May 1948 and for another in October 1948–April 1949. Clumps of beans inoculated with virginoparous apterae from the main, clonal, 'production-line' culture, were placed in a cool but frost-protected glasshouse and screened from all artificial light sources, so that they were exposed to the natural short days of autumn-winter-spring. The parent aphids were removed before their offspring became adult. The latter were mainly apterae but their offspring, the F_2 and later generations, were mainly alatae, with a few apterous viviparae which kept the cultures going. These alatae, unlike the alatae produced at the same time in the main culture exposed to a 16 hr. day, proved very reluctant to feed and produced very few or no young on beans or sugar beet; whereas they fed and produced young readily on spindle leaves, the young developing into oviparae. These alatae were therefore used in the experiments under the provisional title of 'gynoparae'.

During each of the three series of experiments involving gynoparae, two or more samples of both the virginoparous and gynoparous aphids that had been used were caged on spindle leaves in the same greenhouse and their progeny examined as they became adult. The offspring of the aphids from the long-day culture were all viviparous females, mostly apterae. The offspring of the aphids from the cool, short-day cultures were all oviparae, with the exception of a single individual apterous viviparous female found among twenty oviparae from one batch of alatae used in an experiment early in February 1948.

Samples of the aphids were preserved for later counts of the number of secondary 'rhinaria' on the fourth segment of the antennae, a criterion kindly suggested by Dr D. Price Jones in the light of his own (unpublished) studies upon the *A. fabae* complex. The results are shown as frequency distributions in Fig. 1, which also includes a set of counts on wild-caught summer migrants collected from pruned spindle bushes in June–July 1949 and not used in the experiments. The most striking difference is that between the wild-caught aphids and the greenhouse-reared aphids, taken as a whole. The majority of wild-caught spring and summer virginoparae bore no rhinaria on either of the fourth antennal joints, differing sharply in this respect from the wild-caught gynoparae, the majority of which had more than eight rhinaria on the two fourth antennal joints combined. The two kinds of alatae produced in the greenhouse always differed in the same sense, but much less. The majority in all three samples of the long-day (virginoparous) aphids had at least two rhinaria on the two fourth antennal joints combined, while in two of the three short-day (gynoparous) samples the majority had at most six. The treatments given to the aphids in the greenhouse were merely the most convenient available for producing the different reproductive types; they were not varied in the ordered way that would be necessary to determine the critical conditions for producing aphids of particular reproductive and morphological types. But it is noteworthy that the variation of rhinaria number between different batches of one reproductive type, virginoparae or gynoparae, was not consistently related to day-length or even to the direction of change of day-length.

All the aphids were starved for some hours before use to encourage settling on the leaves offered, but the age and degree of starvation of two aphid forms being compared often differed considerably. When the aphids were being produced slowly they had to be accumulated over more than one day to provide enough for one experiment. But the large number of preference and fecundity experiments so far done on *A. fabae* specimens of widely differing ages and degrees of starvation and previous exercise have provided no evidence that these factors can reverse a preference or fecundity difference between different leaves; although the aphids' activity and hence the apparent strength of their preference may be affected. Details of most of the plant material used have already been given by Kennedy & Booth (1951), of the system for classifying leaves according to age by Kennedy *et al.* (1950), and of the cages and procedures for the preference and fecundity experiments by

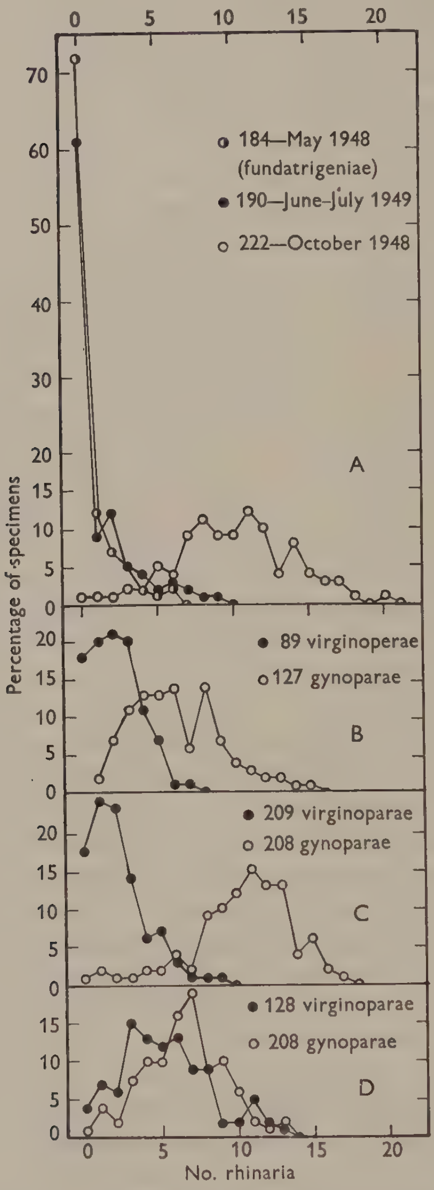

Fig. 1. Frequency distributions of the numbers of secondary rhinaria on the fourth segments of the two antennae combined, of the winged females used in the experiments. A, field collections from *Euonymus europaeus*. B–D, greenhouse cultures on *Vicia faba*: B, February 1948; C, April 1948; D, October–November 1948.

Kennedy & Booth (1950). A 'mature' leaf as here understood is a leaf that has ceased growth and acquired its deepest green colour; it is not a leaf that has begun visible senescence.

COMPARISON OF DIFFERENT VIRGINOPAROUS FORMS

Feeding preferences

Table 1 summarizes the results of the first series of experiments on preferences between spindle and sugar-beet leaves, in January 1948. Ten apterous and ten alate virginoparae from the 16-hr.-day culture were introduced together into two-leaf cages on pot plants in the greenhouse, and one distribution count taken some hours later. Both aphid forms showed a general preference for spindle over beet. The apterae showed this preference strongly only in group B, where the spindle leaves were at a more acceptable physiological age (growing) than the beet leaves (mature). When the two kinds of leaves were at about the same physiological age (groups A and D), and when the beet leaves were more acceptable in this respect than the spindle (group C), the two kinds of leaves were favoured almost equally often by the apterae. The alatae, on the other hand, showed their preference for spindle leaves clearly in all comparisons, always more consistently than the apterae. As with the apterae the preferences of the alatae for spindle was strongest when the spindle leaves were younger than the beet leaves. But they preferred even mature spindle leaves to young beet leaves in these experiments where the spindles were well-watered and rather soft owing to the warmth and winter light. The indications were, therefore, that leaf age played more part in aptera than in alata preferences, while plant kind played more part in alata than in aptera preferences.

TABLE 1. *Preferences of cultured apterous and alate virginoparae offered a choice between the same pairs of spindle and beet leaves*

	Leaves compared		No. of leaf pairs	Total presentations	Aphid forms	Simple majorities	
Exp.	Spindle	Beet				Spindle	Beet
A	Young	Young	10	14	Apterous	8	6
					Alate	9	4
B	Young	Mature	22	24	Apterous	18	4
					Alate	21	2
C	Mature	Young	10	14	Apterous	7	5
					Alate	10	4
D	Mature	Mature	4	8	Apterous	3	5
					Alate	7	1
		Total	46	60	Apterous	36	20
					Alate	47	11

The second series of experiments with apterous and alate virginoparae from the 16-hr.-day culture, given a choice between spindle and beet leaves, was done in October 1951, on plants in the outdoor raised bed described by Kennedy & Booth

(1951). Only one type of leaf was used from each kind of plant to permit more replication than in the earlier experiments. The types of leaf used, mature from spindle and young-mature from beet (*M* and *YM* of Kennedy *et al.* 1950), were chosen as being likely to show most clearly the difference between the preferences of the two aphid forms. So long as the two forms showed a preference for the same kind of leaf in experiments of this type, it was possible to demonstrate differences only in the strength or degree of their preferences, not in the sense of them. But if it were true that alatae were more sensitive to leaf kind and apterae to leaf age, then, when offered a leaf of the preferred kind at an unacceptable age, alongside a leaf of the less acceptable kind at somewhat more acceptable age, the alatae might be expected to show a preference for the more acceptable *kind* of leaf (spindle) while the apterae might prefer the other kind of leaf (beet) because it was more acceptable in age.

TABLE 2. *Preferences of cultured apterous and alate virginoparae offered a choice between the same pairs of mature spindle and young-mature beet leaves* (*twenty leaf pairs and thirty presentations*)

	Wins		Simple majorities	
Aphid form	Spindle	Beet	Spindle	Beet
Apterous	2	17	4	26
Alate	9	2	16	14

TABLE 3. *Preferences of wild-caught apterous and alate fundatrigeniae offered a choice between the same pairs of spindle and beet leaves*

Leaves compared		No. of leaf pairs	Total presentations		Simple majorities	
Spindle	Beet			Aphid form	Spindle	Beet
Young	Mature	6	25	Apterous	25	0
				Alate	25	0
Mature	Young	5	25	Apterous	12	11
				Alate	22	3

Ten such two-leaf cages were used simultaneously. The two aphid forms were introduced into one cage alternately, so that apterae were tested in five of the cages in the morning and alatae in the same cages in the afternoon, and vice versa for the other five cages. The 'standard' experimental procedure (see Kennedy & Booth, 1950) was followed, with thirty individuals introduced into each cage, four distribution readings at 20 min. intervals and final classification of the results into *wins* for either leaf, or *draws*, as well as into the numbers of occasions on which a simple majority of aphids were found on either leaf. The two aphid forms did in fact show opposite preferences in these experiments (Table 2). The apterae preferred the beet leaves very consistently, while the alatae preferred the spindle leaves although somewhat less consistently.

Alate and apterous virginoparae, but this time both wild-caught fundatrigeniae, were offered a choice between spindle and beet leaves in May 1950. The aphids were taken directly from spindle leaves in the garden, sorted and starved for a few hours. Ten individuals of each were then introduced together into two-leaf cages presenting two combinations of leaves: young spindle with mature beet, and mature spindle with young beet, all on plants in the outdoor raised bed. One distribution reading was taken about an hour later. Fresh aphids were introduced and further readings taken several times with each caged leaf pair in the course of 5 days. The two aphid forms showed an equally strong preference for young spindle over mature beet leaves (Table 3). The alatae showed a hardly less marked preference for mature spindle over young beet leaves. Such behaviour is not typical (Kennedy *et al.* 1950; Kennedy & Booth, 1951) and may have been due to the dull wet weather. The significant point, however, is that the apterae, offered the same leaf pairs under the same conditions, behaved differently, showing a preference for the beet almost as often as for the spindle leaves. Though these alatae were destined very soon to leave the spindle plants and colonize for the most part quite different, herbaceous, secondary hosts including sugar beet itself, yet they showed a stronger preference for the spindle leaves than did the apterae which were to stay out their lives there.

TABLE 4. *Preferences of cultured alate virginoparae and wild-caught alate fundatrigeniae offered a choice between the same pairs of spindle and beet leaves*

Leaves compared		No. of leaf pairs	Total presentations		Wins		Simple majorities	
Spindle	Beet			Aphid form	Spindle	Beet	Spindle	Beet
Young	Mature	9	12	Al. cult.	9	2	9	3
				Al. fund.	12	0	12	0
Mature	Young	9	12	Al. cult.	0	9	1	11
				Al. fund.	2	8	3	9

The apterous and alate fundatrigeniae from spindle thus differed from one another, in their preferences, in the same way as the apterous and alate virginoparae from the 16-hr.-day greenhouse culture on beans. Alatae from these two sources were compared directly in spindle-beet preference cages on the outdoor raised bed in May 1948. The two forms were introduced alternately, not together, into the same cages, and the standard procedure followed. Both kinds of alata preferred the young spindle to the mature beet leaves and the young beet to the mature spindle leaves (Table 4). If they differed at all, the fundatrigeniae showed a slightly stronger preference for spindle over beet than the alatae from the 16-hr.-day culture.

In order to show directly that leaf age plays a more important part in the preferences of apterae than of alatae, they were offered leaves of different ages on the same kind of plant. These experiments were done according to the standard

TABLE 5. *Preferences of cultured apterous and alate virginoparae offered a choice between the same pairs of young and mature leaves from one kind of plant*

Plant	Exp.	No. of leaf pairs	Total presentations	Aphid form	Wins		Simple majorities	
					Young	Mature	Young	Mature
Spindle	A	4	12	Apterous	12	0	12	0
				Alate	7	0	9	2
	B	10	30	Apterous	30	0	30	0
				Alate	12	0	30	0
Total		14	42	Apterous	42	0	42	0
				Alate	19	0	39	2
Beet	C	4	12	Apterous	5	0	11	1
				Alate	4	0	9	3
	D	4	12	Apterous	8	0	11	1
				Alate	4	0	12	0
	E	10	20	Apterous	19	0	20	0
				Alate	6	0	19	1
Total		18	44	Apterous	32	0	42	2
				Alate	14	0	40	4
Total, both plants		32	86	Apterous	74	0	84	2
				Alate	33	0	79	6

procedure, using cultured virginoparae on the spindle and beet plants in the outdoor raised bed: Exps. A and C in mid-March 1949; Exp. D in late April 1949; Exps. B and E in September 1951. In Exps. B and E the two aphid forms were introduced alternately into the same cages. In Exps. A, C and D, gynoparae were tested as well as the two forms of virginoparae. The same four two-leaf cages were used on 3 successive days in each experiment, and loaded once with each aphid form separately each day. The three aphid forms were tested in the order *a–b–c* on the first day, *b–c–a* on the second and *c–a–b* on the third. The gynopara results will be discussed later. Table 5 shows that both apterous and alate virginoparae clearly preferred the young to the mature leaves of both spindle and beet: there were no *wins* at all for mature leaves and even simple majorities were few. But in every experiment, the alatae gave fewer *wins* for young leaves than the apterae, and less than half as many when all the experiments are combined.

Reproduction rates

Experiments on the comparative reproduction rates of cultured virginoparae were done on the same leaves as those used for the preference experiments in autumn 1951 (p. 93 and above). As soon as a set of preference experiments had been completed, the ten pairs of leaves were released from the preference cages and a separate single-leaf cage attached to each leaf. Apterae were then introduced, about ten at a time, into five of the ten cages containing each of the two leaf types, and alatae into the other five. The adults were counted daily, and the survivors and the total young produced were counted a few days later when all the cages were emptied. They were then re-stocked with alatae where they had had apterae before, and vice versa, and the same counts repeated. The mean daily output of young by each live parent for all the cages (to which no accident occurred) containing each kind of leaf and aphid form, was calculated separately for each set of concurrent cagings, since the weather and general level of fecundity varied between sets. Because the experiments took longer than the preference experiments, the growing leaves were on the average nearer to maturity than they had been in the preference experiments.

TABLE 6. *Comparative reproduction rates of cultured apterous and alate virginoparae on the same mature spindle and young-mature beet leaves*

		No. of leaves		Young per live parent-day		
Exp.	Aphid form	Spindle	Beet	Spindle	Beet	Ratio, Sp.: Bt.
A 1	Apterous	4	5	1·89	2·04	0·93
	Alate	5	4	3·08	0·57	5·40
A 2	Apterous	5	5	1·19	1·25	0·95
	Alate	5	5	1·66	0·26	6·38
B 1	Apterous	5	5	1·71	1·28	1·34
	Alate	5	5	2·00	1·19	1·68
B 2	Apterous	5	5	1·93	1·58	1·22
	Alate	5	5	1·89	1·32	1·43
Total	Apterous	19	20	Means		1·11
	Alate	20	19			3·72

TABLE 7. *Comparative reproduction rates of cultured apterous and alate virginoparae on the same young and mature leaves from one kind of plant*

		No. of leaves		Young per live parent-day on leaves		
Plant	Aphid form	Young	Mature	Young	Mature	Ratio, $Y{:}M$
Spindle	Apterous	4	4	4·16	2·31	1·80
	Alate	4	4	2·84	2·47	1·15
	Apterous	4	4	2·53	2·06	1·24
	Alate	3	3	1·78	1·36	1·31
Beet	Apterous	5	3	1·17	0·55	2·13
	Alate	4	5	3·20	1·69	1·89
	Apterous	5	5	0·63	0·36	1·86
	Alate	5	5	2·01	1·07	1·89
Total, both plants	Apterous	18	16	Means		1·76
	Alate	16	17			1·56

Table 6 summarizes the results of the experiments on comparative reproduction rates as between spindle and beet leaves: the same leaves as used in the preference experiments summarized in Table 2. The ratio of the young-production rate on spindle, to that on beet, was always higher for alatae than for apterae. The alatae always produced more young on spindle than on beet, even up to six times more

(Exp. A 2), while the apterae sometimes produced rather more young on beet than on spindle (Exp. A 1 and 2).

Table 7 summarizes the results of experiments on young and mature leaves from one kind of plant: the same spindle leaves as used in the preference experiments summarized in Table 5, Exp. B, and the same beet leaves as used in the Table 5, Exp. E. In contrast with the previous experiments on leaves of different kinds, the comparative reproduction rate of the apterae on the two ages of leaf did not differ consistently or much, from that of the alatae.

COMPARISON OF VIRGINOPARAE AND GYNOPARAE

Feeding preferences

These experiments were done with the three separate stocks of gynoparae described under 'Materials' (pp. 90–1). The three stocks, and so the periods in which the experiments were done, are indicated in the tables of results here as 'Gyn. I', 'Gyn. II' and 'Gyn. III'. Experimental procedures were sometimes modified on account of the irregular and restricted supply of these aphids. Table 8 summarizes the preference experiments as between spindle and beet leaves of various ages. Exps. A, C, H and I were done on the outdoor raised bed, and Exps. B, D, E, F and

TABLE 8. *Preference of alate virginoparae and gynoparae offered a choice between the same pairs of spindle and beet leaves*

	Leaf types compared		No. of leaf pairs	Total presentations	Aphid form	Wins		Simple majorities	
Exp.	Spindle	Beet				Spindle	Beet	Spindle	Beet
A	Young	Young	3	5	Virg.	3	1	4	1
					Gyn. I	0	0	3	2
B	Young	Mature	4	18	Virg.	9	2	11	6
					Gyn. III	9	2	13	5
C	Young	Senescent	3	4	Virg.	2	1	2	2
					Gyn. I	2	0	4	0
D	Mature	Young	8	8	Virg.	0	0	0	6
					Gyn. I	4	0	7	1
E	Mature (soft)	Young	4	24	Virg.	16	0	19	4
					Gyn. III	20	0	24	0
F	Mature (wilted)	Young	4	4	Virg.	0	2	0	4
					Gyn. III	1	2	1	3
G	Mature	Mature	6	10	Virg.	5	2	9	1
					Gyn. III	8	0	10	0
H	Senescent	Mature	6	10	Virg.	3	1	8	2
					Gyn. II	3	1	8	2
I	Senescent	Senescent	7	12	Virg.	3	1	7	5
					Gyn. II	7	1	11	1
	All		45	95	Virg.	41	10	60	31
					Gyn.	54	6	81	14

G on pot plants in the greenhouse. In Exps. B, D, E and G only one distribution reading instead of the standard four was taken after each loading of the cages, and the aphids were then carefully collected, mixed and re-used instead of being discarded and only fresh ones used for each loading. The results fell into the same pattern as those in Tables 1–3, with the gynoparae differing from the alate virginoparae in much the same way as the alate virginoparae differed from the apterous virginoparae. Both forms showed a general preference for spindle over beet but the gynoparae more strongly than the alate virginoparae. When fairly 'hard' mature spindle leaves were offered against growing beet leaves (Exp. D), the virginoparae preferred beet but the gynoparae still preferred the spindle leaves. When the mature spindle leaves were made even less acceptable by denying the plants water until they wilted (Exp. A), the gynoparae shifted over to preferring beet, but still not as strongly as the virginoparae.

TABLE 9. *Preferences of apterous and alate virginoparae and of gynoparae offered a choice between the same young and mature leaves from one kind of plant*

Plant	Leaf pairs	Total presentations	Aphid form	Wins		Simple majorities	
				Young	Mature	Young	Mature
Spindle	2	6	Al. virg.	3	0	5	1
			Gyn. III	0	2	1	4
	4	12	Ap. virg.	12	0	12	0
			Al. virg.	7	0	9	2
			Gyn. III	4	4	7	5
Beet	4	9	Ap. virg.	5	0	9	0
			Al. virg.	3	1	7	2
			Gyn. III	2	2	4	5
	4	12	Ap. virg.	8	0	11	1
			Al. virg.	4	0	12	0
			Gyn. III	0	0	8	1
Total, both plants	14	39	Al. virg.	17	1	33	5
			Gyn.	6	8	20	15

Experiments on young and mature leaves from one kind of plant are summarized in Table 9. The first of these was done in the greenhouse in November 1948, using the same very 'soft' spindle plants as used for Exp. E in Table 8. The other three experiments in Table 9 were the ones done with three aphid forms simultaneously—apterous and alate virginoparae and gynoparae—as already described on p. 96. In all four experiments in Table 9 the virginoparae showed a stronger preference for the young leaves than did the gynoparae. In the first spindle experiment the gynoparae actually preferred the mature leaves which, being soft, were already known to be more acceptable than normal mature leaves. In the second spindle and first beet experiments, the gynoparae settled indifferently on young and mature leaves, while the alate virginoparae showed a preference for the young leaves which the apterae also preferred but more strongly. In the second beet experiment, all three forms showed a preference for the young leaves, but this preference was weak in the gynoparae, stronger in the alate virginoparae and strongest in the apterae.

Reproduction rates

The restricted supplies of gynoparae precluded the use of exactly the same leaves for the fecundity experiments as for the preference experiments. Exps. B, D, G and H in Table 10 were done separately from any preference experiments with the given types of leaf: Exps. B and G in the greenhouse in November 1948 using the very soft spindle plants, and Exps. D and H in the greenhouse in March 1948 and 1949 respectively. Exps. A, C, E, F and I in Table 10 were done concurrently with, and on leaves chosen for close resemblance to, those used in preference Exps. A, B, E, F and I, respectively, in Table 8. The results again paralleled the comparisons between the apterous and alate virginoparae. The ratio of the young-production rate on spindle to that on beet was always greater with the gynoparae than with the alate virginoparae, sometimes several times greater. This was mainly because the gynoparae consistently fed little and produced very few young on the beet leaves. In Exps. A, D, E and F the virginoparae produced more young on the (growing) beet leaves than on the spindle leaves, but the gynoparae, vice versa. Even when

TABLE 10. *Comparative reproduction rates of alate virginoparae and gynoparae on similar sets of spindle and beet leaves*

	Leaf types compared			No. of leaves		Young per live parent-day		
Exp.	Spindle	Beet	Aphid form	Spindle	Beet	Spindle	Beet	Ratio Sp.: Bt.
A	Young	Young	Virg.	2	2	0·77	1·59	0·48
			Gyn. I	2	2	0·68	0·09	7·56
B	Young	Young	Virg.	4	6	2·09	1·11	1·88
			Gyn. III	4	7	0·62	0·17	3·65
C	Young	Mature	Virg.	3	3	2·11	0·73	2·89
			Gyn. III	2	2	0·70	0·15	4·67
D	Mature	Young	Virg.	3	3	0·70	1·90	0·37
			Gyn. I	3	3	2·50	1·20	2·08
E	Mature (soft)	Young	Virg.	7	6	0·99	1·11	0·89
			Gyn. III	7	7	0·66	0·17	3·88
F	Mature (wilted)	Young	Virg.	2	3	0·80	6·20	0·13
			Gyn. III	3	3	1·00	0·60	1·68
G	Mature	Mature	Virg.	3	3	1·20	0·73	1·64
			Gyn. III	3	2	0·90	0·15	6·00
H	Mature	Senescent	Virg.	6	5	1·63	1·16	1·41
			Gyn. III	5	5	1·14	0·15	7·59
I	Senescent	Senescent	Virg.	11	11	1·12	0·69	1·62
			Gyn. III	9	9	0·79	0·19	4·16
All			Virg.	41	42	Means		1·26
			Gyn.	38	40			4·59

the spindle leaves were not only mature but wilted and the beet leaves growing (Exp. F) and the virginoparae were producing more than six times as many young on the beet as on the spindle leaves, the gynoparae still produced more young on the spindle than on the beet leaves.

TABLE 11. *Comparative reproduction rates of alate virginoparae and gynoparae on similar sets of young and mature leaves from one kind of plant*

		No. of leaves		Young per live parent-day		
Plant	Aphid form	Young	Mature	Young	Mature	Ratio, *Y*:*M*
Spindle	Virg.	3	3	2·11	1·20	1·76
	Gyn. III	2	3	0·70	0·90	0·78
	Virg.	2	4	2·04	0·83	2·46
	Gyn. III	2	4	0·54	0·48	1·12
Beet	Virg.	3	3	0·88	0·73	1·20
	Gyn. III	3	2	0·10	0·15	0·67
Total, both plants	Virg.	8	10	Means		1·81
	Gyn.	7	9			0·86

The experiments providing the results given in Exps. B, C, E and G of Table 10 involved the simultaneous comparison of young and mature leaves of spindle and beet, and so provide also some data for the comparison of young and mature leaves on one kind of plant. These are given in Table 11 and again show a clear difference between the two aphid forms. The alate virginoparae reproduced consistently faster on the growing than on the mature leaves, and the ratio between their reproduction rates on these two ages of leaf was consistently greater than that of the gynoparae. The plants were soft, and in two comparisons the gynoparae actually produced more young on the 'mature' than on the growing leaves, although this may not be significant.

DISCUSSION

First, a technical point. The strength of the preference aphids show for one of two leaves in a cage depends on their degree of activity, and that in turn depends on the treatment they received before being offered the choice (p. 91 and Kennedy & Booth, 1950). The pre-treatments given to the different aphid forms whose preferences were compared in the present experiments were not standardized. In the experiments showing a difference only in the strength of the same preference of two aphid forms, this difference may therefore have been partly due to a difference in activity arising from different pre-treatment, rather than to an inherent difference between the two forms. But there are reasons for regarding activity differences as of minor importance here. In the majority of the comparisons made, some experiments showed not a mere difference in the strength of the same preference, but opposite preferences, with one aphid form preferring one leaf and the other form the other leaf when a particular combination of leaves was offered to them. That is not easily attributable to a difference in preconditioned activity, and indicates an intrinsic difference between the two forms. Moreover, those particular combinations of leaves which revealed opposite preferences in two aphid forms, were the ones expected to do so from the pattern of results obtained with the other combinations of leaves which gave only a difference in the strength of the same preference. All the comparisons, whether they showed opposite preferences or different strengths of the same preference, fell into a single pattern referable to the kind and condition of the leaves and the form of aphid to which they were offered.

All three aphid forms showed a preference for *Euonymus* over sugar beet, but this preference was much the strongest in the gynoparae, weaker in the alate virginoparae and weakest in the apterous virginoparae. The order was reversed with leaf-age preferences. All three forms showed some preference for growing over mature leaves of the same kind (see also Kennedy *et al.* 1950), but this preference was strongest in the apterae, weaker in the alate virginoparae and weakest in the gynoparae.

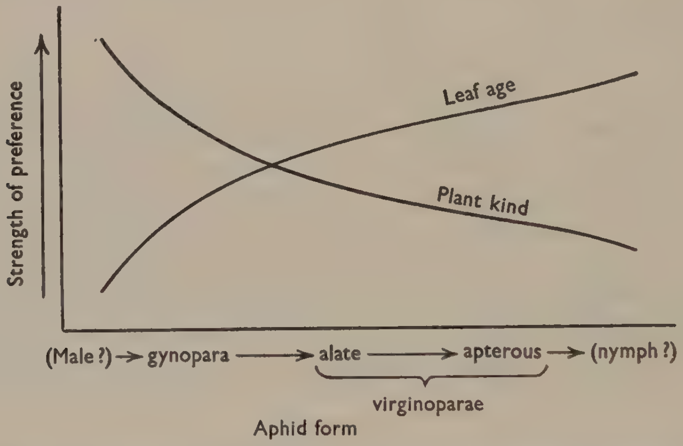

Fig. 2. Schematic representation of the variation among the different forms of *Aphis fabae*, in the strength of the preferences they show between leaves of different ages (young and mature) on one kind of plant, and between leaves on different kinds of plant (spindle and beet).

Physiological significance

The three forms thus fall into a series represented schematically in Fig. 2, which at the same time suggests how their differences may be interpreted in terms of sensory physiology. The dual discrimination theory of aphid host selection (Kennedy & Booth, 1951) postulated that the response of settling down to feed depended on the joint action of two classes of stimuli from the plant: 'flavour' stimuli characteristic of the particular kind of plant, and 'nutrient' stimuli characteristic of the condition and especially the age of leaf, and closely associated or identical with the materials serving for the actual nutrition of the aphids. It is a small step to substitute 'flavour' for 'plant kind' and 'nutrient' for 'leaf age' in the diagram, so postulating that the aphid forms differ in their relative sensitivities to the two classes of stimuli provided by plants. We then have an hypothesis of simple quantitative differences in sensory physiology as the basis for the observed behavioural differences which, in the field, may be sharp and qualitative. The supposition is that the feeding response of the gynopara depends more on flavour than on nutrient stimulation. In the alate virginopara the two are more equal; while the apterous virginopara depends less on flavour than on nutrient stimulation. The gynopara will accept a poor nutrient supply provided the right flavour is strong; the aptera will accept a poor flavour provided the nutrients are rich.

The previous experiments on the alate virginoparae alone (Kennedy & Booth, 1951) showed that, of two leaves offered, the one preferred for feeding was also, as a rule, the one on which more young were produced. But this was less often true when leaves differing in kind were being compared, than when leaves differing only in age were being compared. This greater discrepancy between behaviour and fecundity was interpreted, on the dual discrimination theory, as due to the greater difference of flavour involved in comparisons between two plant kinds. The greater difference of non-nutrient flavour will affect behaviour more than fecundity and so tend to increase the discrepancies between preferences and relative fecundity. On the same reasoning one would expect differences in the relation between behaviour and fecundity when aphids with differing sensitivities to flavour and nutrients are tested on the same set of leaf-pairs. Thus in experiments confined to a set of leaves which differ consistently in kind but inconsistently in age, the fecundity ratio, spindle : beet, should correspond most closely with the strength of the preference for spindle over beet, in the aphid form most sensitive to flavour and least to nutrients: the gynopara. The correspondence should be least in the aptera which is most sensitive to nutrients and least to flavour. This appeared to be so: compare Table 6 with Table 2, and Table 10, Exps. A, C, E, F and I with Table 8, Exps. A, B, E, F and I.

Similarly, in experiments confined to a set of leaves which differ consistently in age but not at all in kind, the fecundity ratio, $Y:M$, should correspond most closely with the strength of the preference for Y over M in the aphid form least sensitive to flavour and most to nutrients, the aptera. The correspondence should be least in the gynopara. This also appeared to be so: compare Table 7 with Table 8, Exp. B and E, and Table 11, Exp. E with Table 9 (first experiment). The gynoparae actually preferred (soft) M spindle leaves to growing ones on which they reproduced somewhat faster. This brings further support for the proposition that leaves differing only in age differ also in their content of specific plant flavour, nutrient and flavour concentrations varying in opposite senses through the life of a leaf with flavour at a maximum at maturity (Kennedy & Booth, 1951). The aphid form more demanding of flavour would then prefer leaves nearer to maturity.

On the available evidence it can be inferred only that the *relative* importance of flavour and nutrient stimuli varies through the aphid forms. The simplest hypothesis would be that the three forms have similar nutritional requirements and possibly a similar sensitivity to nutrient stimulation, differing only in their sensitivity to flavours. As a description of the physiological situation, Fig. 2 would then be incorrect for the nutrient curve would be a straight horizontal line. That, like the nature of the responses themselves, is a matter for further investigation. But the fact that the differences of host preference among the aphid forms are merely quantitative seems characteristic of aphid biology in general. The rhinaria counts given earlier show this feature, as does the frequent appearance of forms intermediate between alatae and apterae (e.g. Davidson, 1927*b*; Bonnemaison, 1951), and the fact that one form of aphid, even a single individual, may produce two or more kinds of offspring, winged and wingless, virginoparous and gynoparous, sexual and asexual (e.g. Davidson, 1921; Bonnemaison, 1951). Neither in morphology, reproductive physiology nor sensory physiology can the forms of an aphid be regarded as qualitatively distinct. Ecologically, they become so.

Ecological significance

It is not difficult to see how the different ecological functions of the seasonal aphid forms may be served by their differing host preferences, as anticipated in the Introduction. The apterous virginopara lies at one end of the series, unencumbered with flight apparatus, more fecund than the alata and specialized as the main food-supply exploiter and vegetative multiplier of the species. Correspondingly, it is behaviourally least demanding in the matter of flavour and most demanding of nutrients. The alate virginopara has the same food-exploiting function as the aptera but carries it out less efficiently. Its anatomical and physiological resources are partially diverted to the flight apparatus which it uses for migration (in the broad biological sense, not in the special sense current among aphidologists) while the aptera stays to feed on the best supply immediately to hand. Migration is part of the alata's second function, of finding fresh plants for exploitation, selecting from among those it encounters the particular kinds to which the aphid species has become adapted. Among these the primary host ranks high, but not as high as many other plants so long as they provide better food supplies for exploitation. Correspondingly, the alate virginopara is rather more discriminating than the aptera in the matter of specific plant flavours, and rather less over nutrients. The gynopara's function is also to reach and select fresh plants, but now less in a foraging than a 'homing' sense. It must colonize the very few species of plants on which the sexuales and their progeny have remained specialized for their part of the cycle. Correspondingly, the gynopara is even more discriminating over specific plant flavours, and less over nutrients, than the alate virginopara.

It may be anticipated that the males which have less need of food than the gynoparae since they bear no young, would prove even more demanding of flavour and indifferent to nutrient stimuli. According to Hille Ris Lambers (1946) the males of *Myzus persicae* have, in fact, a narrower host range than the gynoparae of the species. The same expectation holds *a fortiori* for autumn migrants which do not need to feed or even 'taste' (probe into) the leaves of the winter host before they will deposit their sexual young. Such migrants include, according to Hille Ris Lambers (in a letter he kindly permits me to quote), the gynoparae of *Pemphigus bursarius* (L.) and *Thecabius affinis* (Kltb.), which congregate on *Populus italica* after the leaves have fallen. 'Nutrient' stimulation would seem to have no part in the host selection here, and the 'flavour' stimulation can hardly be gustatory for most of the immigrants. Perhaps this host selection is mainly olfactory and partly tactile, and it is probably aided by gregariousness.

That the differences between the host preferences of the seasonal forms of *Aphis fabae* are essentially quantitative, and not complete reversals as events in the field might suggest, is borne out by observations of particular forms occurring exceptionally on 'wrong' hosts (Mordvilko, 1936; Davidson, 1921; de Fluiter, 1949; Kennedy *et al.* 1950). The reason why the host relations of the forms generally differ quite sharply in the field is that the changes in their preferences are adapted to and supplement the seasonal changes in the environment. In the autumn, the mass senescence of the leaves of the spindle tree is reinforced by the strong specific preference for that tree developed by the aphids (gynoparae) produced at that season, doubly assuring the concentration of the aphid population on to spindles. In the spring and summer, the unacceptable mature condition of the spindle leaves is reinforced by the stronger relative preference of alienicolae for growing and senescing leaves, doubly assuring that the aphids colonize plants other than spindle. It is most probable that additional factors multiply these assurances, including gregariousness and differences in plant turgor and in the local weight of attack by natural enemies, which may favour one or other of the alternate host-groups at different seasons. Acting in combination with all these external factors the graded changes in the aphids' sensory physiology issue in sharp, qualitative changes in their host relations.

It has already been suggested (Kennedy *et al.* 1950; Kennedy & Booth, 1951) that aphid species differ from one another in the same way as the forms of *A. fabae* do. In their stricter avoidance of middle-aged leaves on spindle trees and sugar beets, *Myzus persicae* apterae differed from *Aphis fabae* apterae, in the same way as *A. fabae* apterae differed from alatae, and virginoparae from gynoparae, in the present experiments. This may therefore by an important way of evolutionary divergence, not only within species but between them and beyond. For example, divergence into monoecious and heteroecious species, within the same genus, probably means strengthening the preference of the former for the specific flavour of the one host, and that of the latter for nutrients and therewith for the flavours of other plants. Some monoecious species have alternative nutritional adaptations such as longer stylets permitting feeding through bark on stems and roots (see Bonnemaison, 1951) or the power to form galls on the host (Kennedy, 1951 *a*, *b*), or aestivation (Davidson, 1927 *a*). But these developments also occur among heteroecious aphids, and it is their ability to exploit secondary, herbaceous host plants which gives them their important status as agricultural pests.

It is a pleasure to record our debt to Mr H. L. G. Stroyan for many informative discussions.

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WIND DIRECTION AND THE INFESTATION OF BEAN FIELDS BY *APHIS FABAE* SCOP.

By C. E. TAYLOR

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(With Plate 8 and 8 Text-figures)

Bean fields are often attacked by *Aphis fabae* very heavily along the edges: and on a field at Rothamsted in 1948 the sides facing the wind during the primary migration had more colonies than those in the lee of the crop (Johnson, 1950). Additional observations on the distribution of colonies were made on six bean fields at Sutton Bonington in 1950–2.

At Rothamsted in 1948 it was assumed that the more numerous colonies on the windward sides of the field followed a heavier deposition of primary migrants there, though the migrants themselves were not observed on the crop. In 1950–2 the locations of primary migrants, as well as of colonies, were recorded in three out of the six fields observed.

Introduction

Sites

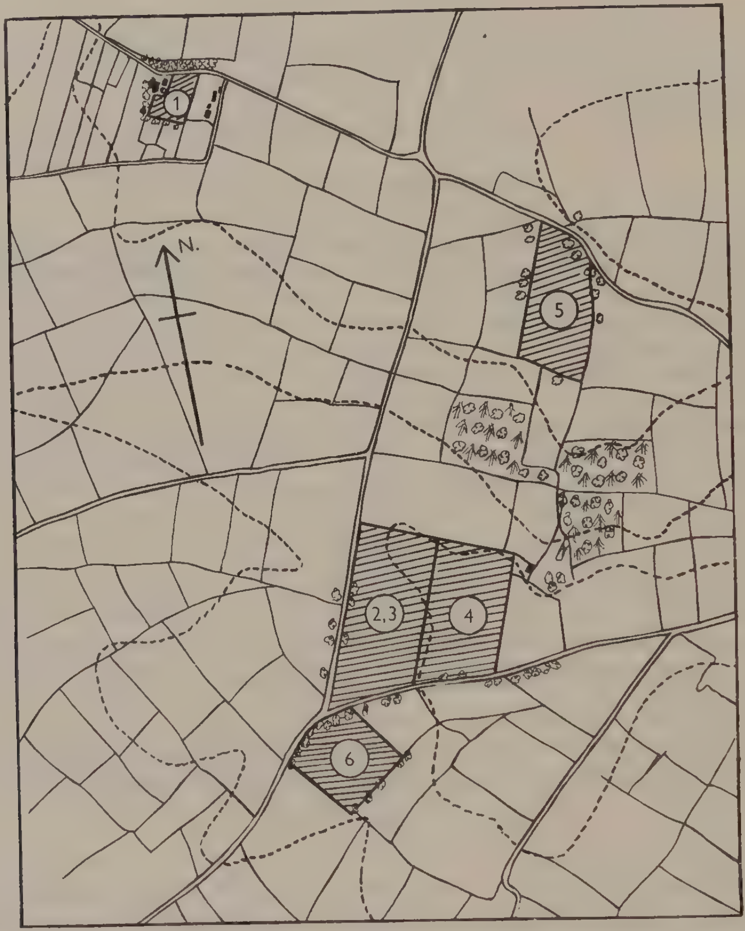
The bean fields, all in the same district near Sutton Bonington (Text-fig. 1), are named as follows in the text: (1) Italdo, 1950; (2) Cold Harbour A, 1951; (3) Cold Harbour A, 1952; (4) Cold Harbour B, 1952; (5) Brickyard Lane, 1952; (6) Grange Farm, 1952.

In (2), (3), (4) and (6), air photographs make lengthy descriptions of the fields unnecessary (Pl. 8); the other fields are described later in the text.

Methods

Sampling for migrants and colonies was usually done only round the edges of the field, partly because bean fields suffer attack especially at the edges and partly owing to lack of time and labour. But three of the fields in 1952 were photographed from the air on 30 July when the infestation had become well established and when final counts had been made round the edges. These photographs show the infestation pattern over the whole of the field, confirm the pattern of the distribution of colonies round the edges, and support the hypothesis that edge-sampling indicated the major *bias* of infestation over the field.

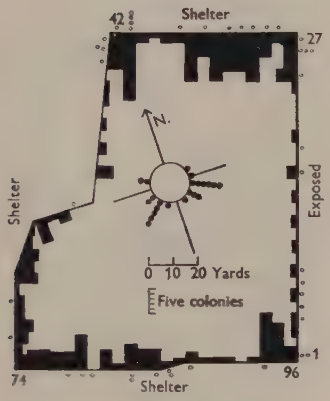
The edges of the field were marked out in sections 5 yd. long and 2 yd. deep into the crop. Two stems were selected at random from each section on each sampling day; the number of migrants and the presence or absence of a colony was recorded, without removing stems from the field. On the last day of the observations, when the final pattern was well established and the summer migration well advanced, the depth of the sections was increased to 3 yd. and *all* colonies 3 in. and more in length were counted in each section.



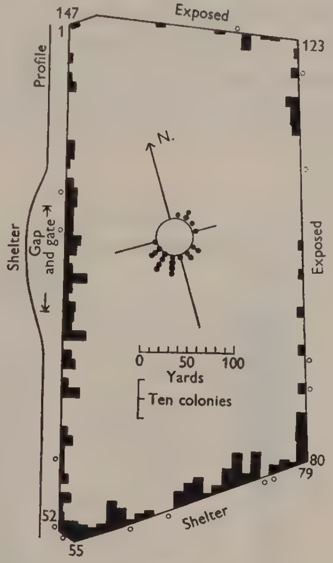
Text-fig. 1. Map of area showing fields sampled in 1950, 1951 and 1952.

The period of primary migration was found by recording the departure of alatae from spindle trees at Sutton Bonington and their arrival on the beans. There were no spindle trees within 1 mile radius of the fields.

Wind direction was observed daily at 09.00 hr. G.M.T. at Sutton Bonington and again at the field between 11.00 hr. and 15.00 hr. G.M.T. The frequency of wind from various directions during the primary migration is indicated by wind roses on Text-figs. 2–7 which were made from combined observations at the field and at the School of Agriculture, Sutton Bonington.



Text-fig. 2.



Text-fig. 3.

Text-fig. 2. Italdo, 1950. Figures at corners of the diagram refer to section numbers. ○, primary migrants; ■, number of infested stems from daily samples. Only edges sampled, not the interior of the field.

Text-fig. 3. Cold Harbour A, 1951. Figures refer to section numbers. Alternate sections were not sampled and are omitted from the diagram. ○, primary migrants; ■, number of infested stems from daily samples. Only edges sampled, not the interior of the field.

WIND DIRECTION AND INFESTATION

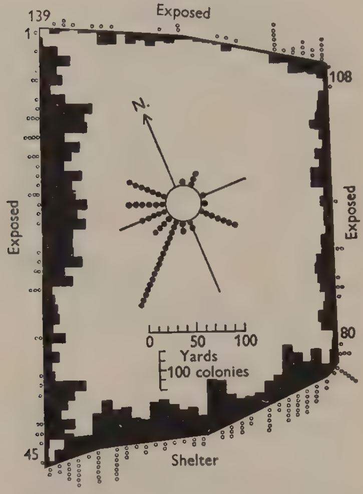
Italdo, 1950 (Text-fig. 2)

This was the only field where infestation showed no clear relation to wind direction.

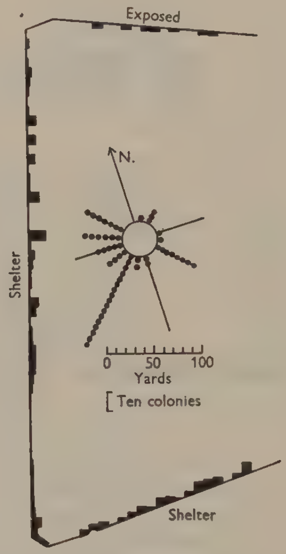
The field was small, with only the east side, adjacent to a pasture, well exposed. There were willows and scattered bushes giving some shelter on the south and south-west sides; bushes and buildings occupied the indent opposite sections 43–62; the north side had a 3 ft. hawthorn hedge backed by a spinney. Beans were spring sown in rows N.N.E. to S.S.W. and were 1–2 ft. high at the primary migration. The crop was patchy but except in sections 57–63 the edges were fairly uniform.

Wind direction and primary migration. Alatae on spindle trees, a small bean plot and the field itself, indicated that migration occurred mainly between 29 May and 9 June, with the peak from 7 to 9 June. Winds for this period were mainly from the south-west and south-east (Text-fig. 2). Migrants were found mainly on the north, east and south sides of the field; this deposition is not clearly related to prevailing wind direction, for there were more on the north side than was expected.

Infestation pattern. Sampling was done daily from 29 May to 23 June and then on alternate days until 10 July. Text-fig. 2 shows the distribution of colonies sampled. The sheltered north side is again the most heavily infested. But the exposed east side which had many migrants is scantily infested while the converse situation occurs on the west. Thus the south and west sides have become relatively well infested as might be expected from the south-westerly winds. But the heavy infestation on the north side and the low infestation on the east side is evidently due not only to wind direction; for although the east side had many migrants as expected, it developed few colonies. The north side had both migrants and colonies but was not exposed directly to wind.



Text-fig. 4.



Text-fig. 5.

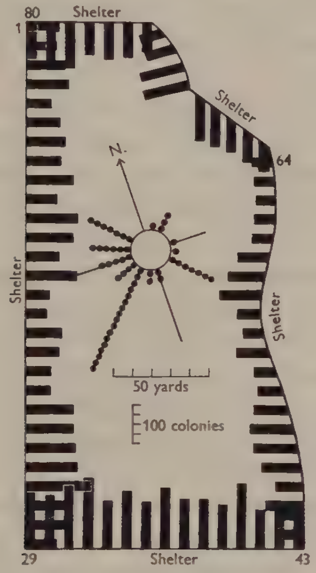
Text-fig. 4. Cold Harbour B, 1952. Figures refer to section numbers. Alternate sections not sampled and omitted from the diagram. ○, primary migrants; ■, number of colonies 3 in. or more in length at final count round edges. For distribution of infestation across the field see Table 2.

Text-fig. 5. Cold Harbour A, 1952. Volunteer beans in wheat. Alternate sections omitted. ■, number of colonies 3 in. or more in length at final count round edges only.

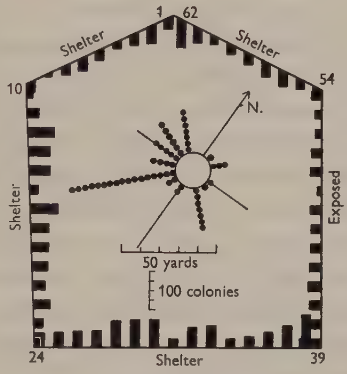
The total number of colonies of 3 in. and more in length recorded on 12 July shows a similar distribution to that of the colonies counted during the period 29 May to 10 July.

Cold Harbour A, 1951 (Pl. 8, fig. 1; Text-fig. 3; Table 1)

This large field is well exposed on high ground. The south side, slightly below the road level, is bounded by a 3 ft. hawthorn hedge with bushes on the opposite side of the road up to 15 ft. high: for about 70 yd. at the east end of the south side oak trees grow on the opposite side of the road. The west side was the most sheltered with a 3 ft. hawthorn hedge and some bushes up to 15 ft. high except at a gap of about 100 yd. (sections 19–20), between two elm trees which contained only the 3 ft. hedge and a gate; there, also, the field dropped to form a wide shallow depression (Text-fig. 3). On the east side there was no shelter and the adjacent field was under wheat, 1 ft. high when migration occurred. The north side, bounded by a cart track, was exposed, with a potato field beyond.



Text-fig. 6.



Text-fig. 7.

Text-fig. 6. Brickyard Lane, 1952. ■, number of colonies 3 in. or more in length at final count around edges only. Alternate sections shown in diagram but not sampled.

Text-fig. 7. Grange Farm, 1952. ■, number of colonies 3 in. or more in length at final count around edges only. Alternate sections shown in diagram but not sampled.

Beans were autumn-sown, 1–2 ft. high during primary migration, in rows north to south with the headlands 5 yd. wide at the north and south ends. Except at the corners, alternate 5 yd. sections only were numbered and sampled.

Wind direction and primary migration. In 1951 migrants were first seen on the field on 29 June, and subsequently on 30 June, 3 and 6 July, four consecutive sampling days.

During 29 June to 6 July, the wind was mainly from the south-west and the south-east quarters and few migrants were observed; but they occurred, as expected, chiefly on the west, south and east sides.

Infestation pattern. From 29 June to 31 July the field was sampled every 4 days. Only 206 of 3822 stems examined were infested; colonies were relatively more numerous on the sheltered south and west sides, especially opposite the gap on the west side, few on the east and very scanty on the north side.

Distribution in this field was related to shelter and the direction of the wind but not precisely to the deposition of primary migrants. Records of these, being very sparse, are liable to large sampling errors (Text-fig. 3).

Cold Harbour B, 1952 (Plate 8, fig. 1; Text-fig. 4; Tables 1 and 2)

This field, adjacent to Cold Harbour A, is well exposed, particularly on the north, west and east sides (see Pl. 8, fig. 1). Beans were autumn-sown in rows north to south, with headlands 5 yd. wide at the row ends. The plants were 1½–3 ft. high during the primary migration. On the south side a 3 ft. hedge bordered the crop, with hawthorn bushes and several oak trees on the opposite side of the road. The remaining sides were exposed (except for a 4 ft. hedge at the north-east corner); cereal crops about 1½ ft. high were adjacent to the west, grass to the east and a cart track and arable land to the north.

Wind direction and primary migration. Primary migrants were observed on spindle trees from 10 to 15 May, but they were not found on the field until 16 May. Thereafter a strong migration continued until 13 June: wind during this period was mainly from the north-west to south-west, with some from the south-east and relatively little from the north and north-east. The abundance of migrants enabled their distribution to be clearly observed. They occurred abundantly on the south and west, less frequently on the north and east sides (Text-fig. 4).

Infestation pattern. In addition to edge sampling, four traverses were made on 8 July, north to south across the field: two, opposite sections 57 and 65; two, 25 yd. from the east and west sides respectively (Table 2). Sections along the traverses were 15 yd. long and 5 yd. wide. An air photograph taken on 30 July shows the final infestation pattern and the surrounding features of the field.

As at Cold Harbour A only alternate sections were used for edge sampling. These, the traverses and the photographs show that the western half of the field and the southern edge bore the heaviest infestation, not only of colonies but also of migrants. The wind during primary migration was directed mainly on these sides. A scatter diagram (Text-fig. 8) of numbers of colonies per 10 sections against the corresponding number of primary migrants, shows a positive relation between deposition of migrants and subsequent colony density, more especially with the samples taken over the whole period rather than with the final 3 in. colony pattern.

Other fields observed in 1952

Maps were made of three other bean fields in 1952 recording only the final infestation pattern along the edges as shown by the total number of colonies 3 in. or more long; migrants were not counted. Wind direction during primary migration was the same as for Cold Harbour B, 1952, Text-fig. 4.

Cold Harbour A, 1952 (Pl. 8, fig. 1; Text-fig. 5, Table 1)

The surrounding features were the same as in 1951. The field was under wheat with a scanty crop of volunteer beans. The wheat and beans were about 15 in. and 1½–2 ft. high respectively during primary migration. Infestation was mapped along the north, west and south sides. As in the adjacent field, Cold Harbour B, 1952, colonies were relatively more numerous on the sheltered south and west and less so on the north. Unfortunately the sides adjacent to Cold Harbour B, 1952 were not mapped. Distribution of colonies on the south, west and north sides corresponded closely to their distribution in 1951.

Brickyard Lane, 1952 (Text-fig. 6; Table 1)

This field, which lies towards the bottom of a shallow valley running east to west, slopes upwards to the south, and is sheltered on all sides by a hedge 4–7 ft. high; on the north-east side especially are several large bushes and trees. The field, which was under spring beans drilled east to west with a headland round the field, was well infested all over, but more heavily along the west and south sides.

Grange Farm 1952 (Pl. 8, fig. 2; Text-fig. 7; Table 1)

This field, of winter beans, broadcast sown, lies at the top of a fairly steep hill and slopes away to the south and west. Along the north-west, north and south-east sides ran hedges about 10 ft. high with several trees, particularly in the north-west side; oats bordered the north-east side, on the south-west a 2 ft. hedge separated the crop from a field of sugar beet.

TABLE 1. *Colony distribution at edges*, 1951 *and* 1952 (*excluding Italdo*)

Side		Westerly	Southerly	Easterly	Northerly
	Number and percentage stems infested in total daily samples				
Cold Harbour A, 1951	No.	1404	650	1144	624
	Percentage	5·9	12·9	2·5	1·6
Cold Harbour B, 1952	No.	4214	2580	2580	2580
	Percentage	21·6	39·0	13·1	10·0
	Mean number of colonies of 3 in. and over per section in final count				
Cold Harbour A, 1951		0·39	1·0	0·19	0·04
Cold Harbour A, 1952		1·7	2·3	0·5	—
Cold Harbour B, 1952		57·4	73·7	29·4	15·2
Brickyard Lane, 1952		99·6	134·3	76·1	92·3
		S.W.	S.E.	N.E.	N.W.
Grange Farm, 1952		41·8	53·1	27·1	31·7

The infestation was heavier on the sides facing south-east and south-west and extended well into the field (Pl. 8, fig. 2). The edge pattern showed a concentration on the south-east and south-west: the north-west side was also relatively well infested. The least infested side was to the exposed north-east.

TABLE 2. *Mean number of colonies of 3 in. and more in length per section along traverses running north to south*

(Cold Harbour B, 1952)

Traverse	No. of colonies
25 yd. from west side	101·4
Between sections 57 and 127	106·5
Between sections 65 and 188	29·9
25 yd. from east side	26·9

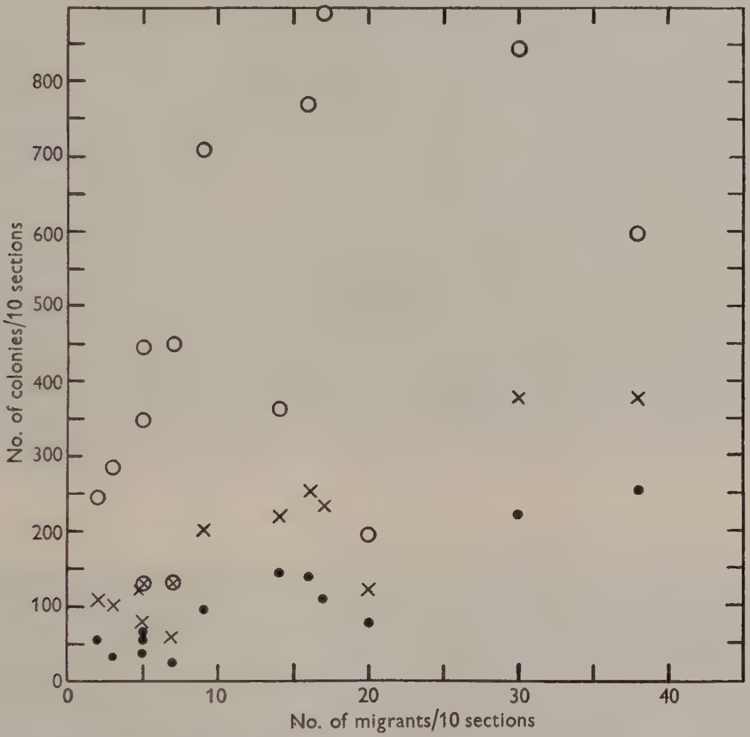
DISCUSSION

Many factors, in addition to wind direction, may influence the pattern of bean-field infestation; for example, shelter or its absence at the edges, or intensity of migration on different days; and variation in the stem density, which has been neglected in this preliminary work. The small number of fields that can be investigated by direct counting methods makes statistical treatment of the problem impracticable in its present stage.

These preliminary studies suggest that the intensity of infestation on different sides of bean fields is associated with shelter and with wind direction during primary migration. But with only a slightly variable wind three sides of a field may be blown against almost equally, so that often out of four sides perhaps three would be relatively well infested rather than one side bearing most of the attack.

In spite of variation from field to field, where winds prevailed from south and west on Cold Harbour A and B, Brickyard Lane, and Grange Farm, the more southerly and westerly sides show greatest infestation; except for Cold Harbour B these sides were also sheltered. The northerly, and after that the easterly, sides, are least infested. Cold Harbour A showed similar patterns in both 1951 and 1952, and compared with Cold Harbour B suggests that a field may regularly show more or less the same pattern. Shelter on the windward side is clearly important: moreover, trees or tall hedges on the *lee* side appear to be associated with some infestation there: e.g. the north side, at Italdo, north and east sides at Brickyard Lane, north-west side at Grange Farm, and where hedges grew at the north-east corners of Cold Harbour B. Broadbent, Tinsley, Buddin & Roberts (1951) noticed that the percentage of virus-infected lettuce plants increased near hedges and trees even when these were on the side furthest from the source of infection: this was probably a reflexion of the same phenomenon.

Where there are more migrants there are usually also more colonies. But there are exceptions (Text-fig. 2). Text-fig. 8 shows the relationship between numbers of primary migrants and numbers of colonies around the edges of Cold Harbour B. In the two lower graphs colonies and migrants were counted on an equal number of stems (2/section/day), so indicating the relation between the numbers of migrants and their effect. For the period of primary migration (16 May–13 June) about seven colonies developed for each migrant found; up to 1 July, approximately ten colonies had developed for every migrant found; the actual proportion of colonies to migrants may be somewhat lower than this for more migrants than colonies may have been missed. In the upper graph in Text-fig. 8 colonies and migrants were not counted on equal numbers of stems and this relationship is not shown.



Text-fig. 8. Cold Harbour B, 1952. Relation between number of colonies and number of primary migrants. ○, total colonies 3 in. or more long at final count; ×, colonies sampled 16 May–1 July inclusive; ●, colonies sampled 16 May–13 June inclusive.

It is a pleasure to thank Dr C. B. Williams for suggestions about the work, F. Taylor, Esq., of Italdo, F. L. Bowley, Esq., of Hall Farm, and W. Evans, Esq., of Grange Farm, for the use of their fields, and those students of the School of Agriculture who made counts.

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EXPLANATION OF PLATE 8

Fig. 1. Air photograph (1200 ft.). The dark field in the centre is Cold Harbour B, 1952. Western half of the field where infestation is heaviest is darker. Adjacent field to the west is Cold Harbour A.

Fig. 2. The dark field in the centre is Grange Farm, 1952 (1200 ft.). The infestation extends well over the field at the south-eastern side. North-east side is comparatively free but south-east and south-west sides show distinct edge infestation.

(*Received* 16 *April* 1953)

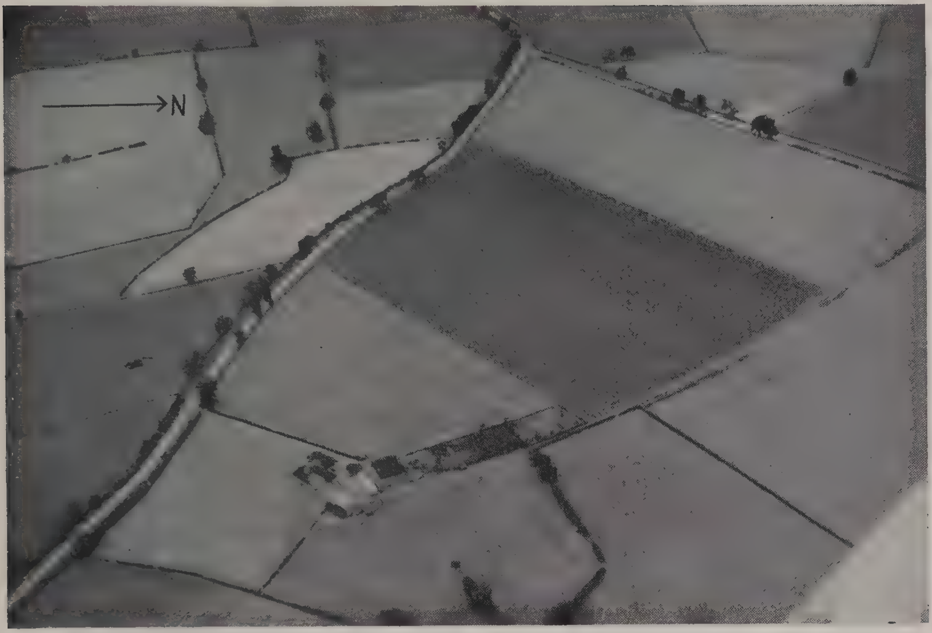

Fig. 1.

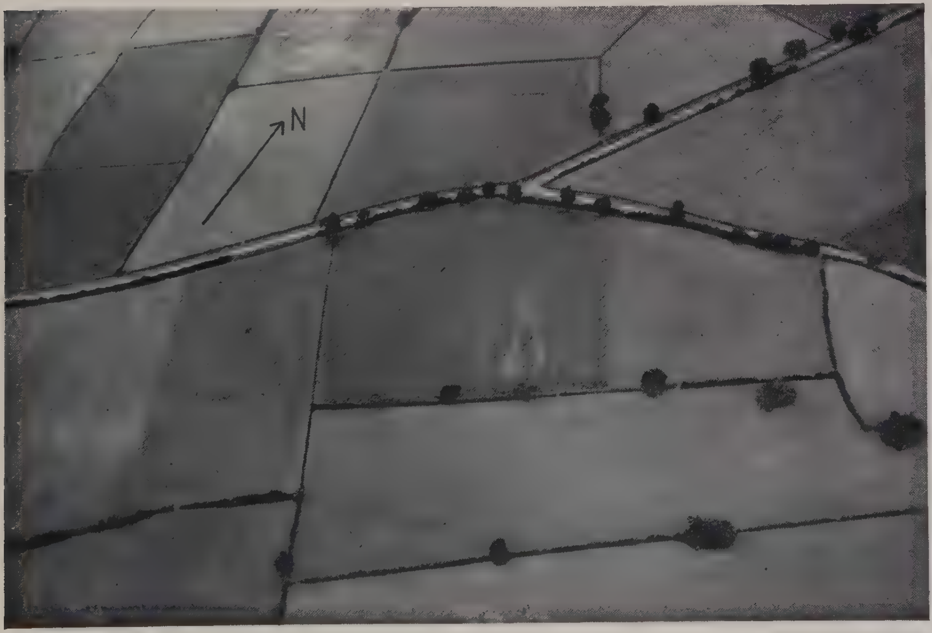

Fig. 2.

TAYLOR & JOHNSON—*Wind direction and infestation of bean fields by* Aphis fabae

Way, M. J., Smith, P. M. & Potter, C. (1954). *Ann. appl. Biol.* **41** (1), 117–131.

STUDIES ON THE BEAN APHID (*APHIS FABAE* SCOP.) AND ITS CONTROL ON FIELD BEANS

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(With 3 Text-figures)

Field trials on chemical control of *Aphis fabae* Scop. attacking spring-sown field beans were carried out during the years 1950–2. The insecticide sprays were applied once only, soon after primary aphid migration into the bean crop had ceased. Destruction of the few aphids present at this early date stops build-up of the big populations that often develop later.

An 0·02 % (w/v) parathion spray was used in all three field trials. This gave excellent control of *A. fabae* as did the systemic insecticides Isopestox and Systox at 0·05 % (w/v) active ingredient, and nicotine at 0·1 % (w/v). Sprays containing allethrin (0·05 % (w/v) pure material), pyrethrins (0·05 % and 0·02 % (w/v) total pyrethrins plus piperonyl butoxide) and an 0·1 (w/v) DDT emulsion gave moderate control. *A. fabae* was not controlled in plots treated with an 0·1 % (w/v) DDT crystalline suspension, and the aphid population reached a higher peak than in the untreated plots; the suspension is not only relatively ineffective against the aphid, but is more toxic than the DDT emulsion to its Coccinellid predators.

On the bean plant the 0·05 % (w/v) Systox spray showed some residual toxicity to *A. fabae* for at least 5 days after application, but stopped causing 100 % kill within 24 hr. 0·02 % (w/v) parathion and 0·1 (w/v) DDT emulsion had slight, and 0·02 % (w/v) pyrethrins and 0·1 % (v/v) nicotine, no residual toxicity.

In the year 1950, aphid attack on the field plots was slight and damage insignificant. In 1951, there was a moderate attack and the yield of bean seed was significantly increased by all insecticidal treatments; where aphid control was most efficient (parathion and Isopestox treatments) the yield (16·7–17·1 cwt./acre) was about × 2·7 that of the untreated control (6·3 cwt./acre). In 1952, there was severe aphid attack, and the mean seed yield of the untreated control plots was 1·4 cwt./acre. Plots treated with the DDT suspension yielded 1·3 cwt. seed/acre, but all other insecticides, including the DDT emulsion, increased the seed yield to 10·3–14·8 cwt./acre.

All preparations used in 1952, except nicotine, were destructive to adult Coccinellid predators of *A. fabae*; *Adalia bipunctata* was more susceptible than *Coccinella septempunctata*. However, in 1952, predators on the experimental area were too uncommon to be economically important.

Introduction

In Britain the acreage planted with field beans, a valuable protein crop, has declined greatly during the last eighty years. There are many reasons for this, one being the serious damage often caused by *Aphis fabae* Scop., which even as small infestations may greatly reduce yield of spring-sown beans (Judenko, Johnson & Taylor, 1952).

Control using nicotine has been suggested, but there are no quantitative data on its efficiency, nor is it known how often, under field conditions, the increased yield would repay successful control. Also *A. fabae* damage does not become obvious until the beginning of July when the bean crop is full grown and liable to be damaged by normal farm spraying machinery. Thus the field control of *A. fabae* on beans is seldom attempted.

This paper describes work carried out during the years 1950, 1951 and 1952 on control of *A. fabae*. In field trials the efficiency of some new insecticides was compared by assessing the bean aphid population on each plot before, and at intervals, after spraying; the nature of aphid damage to beans was studied and seed yields of all plots were recorded. Thus data were obtained on crop loss in relation to *A. fabae* population.

To reduce aphid damage to the minimum the insecticides were applied at an early date before attack became obvious. It was considered that early spraying would also lessen the damage by tractor-drawn spraying machinery.

Aphid predators, notably Coccinellidae, are sometimes common on bean crops infested with *A. fabae*. They were counted and observations were made on their resistance to the insecticides.

Some aspects of the early experiments are summarized by Potter (1952*b*).

MATERIALS

The following insecticide sprays were made up in water:

(*a*) *DDT suspension.* 0·1% (w/v) technical DDT (80% p.p. isomer) in a proprietary product containing a suspension of approximately $1 \times 6\,\mu$ DDT crystals plus 0·1% (w/v) sulphonated lorol in year 1950, and 0·2% (v/v) Lissapol N in year 1952.

(*b*) *DDT emulsion.* 0·1% (w/v) crude DDT (m.p. 95·5–99° C.) plus 0·3% (v/v) 50:50 benzene-toluene mixture and 0·2% (v/v) Lissapol N.

(*c*) *Nicotine.* 0·1% (v/v) nicotine alkaloid (95–98% pure) plus 0·1% (w/v) sulphonated lorol in year 1950 and 0·2% (v/v) Lissapol N in years 1951–2.

(*d*) *HETP.* 0·1% (v/v) technical hexaethyltetraphosphate (containing 20% (w/v) tetraethylpyrophosphate) plus 0·1% (w/v) sulphonated lorol.

(*e*) *Parathion.* Year 1950—0·02% (v/v) proprietary parathion (containing 20% diethyl *p*-nitrophenyl thiophosphate) plus 0·1% (w/v) sulphonated lorol. Years 1951–2—0·02% (w/v) technical parathion (containing 84% diethyl *p*-nitrophenyl thiophosphate and 13% other insecticidal materials plus 0·03% (v/v) 50:50 benzene-toluene mixture and 0·2% (v/v) Lissapol N.

(*f*) *Allethrin.* Technical product containing 80% pure allethrin diluted to 0·05% (w/v) pure material plus 0·2% (v/v) Lissapol N.

(*g*) *Pyrethrins.* Extract of natural pyrethrins (containing 14% (w/v) pyrethrin I and 11% pyrethrin II). Year 1951—0·05% (w/v) total pyrethrins plus 0·05% (v/v) piperonyl butoxide and 0·2% (v/v) Lissapol N. Year 1952—0·02% (w/v) total pyrethrins plus 0·2% (v/v) piperonyl butoxide, 0·2% (v/v) 50:50 benzene-toluene mixture and 0·2% (v/v) Lissapol N.

(*h*) *Isopestox*. Technical product containing 90% bis-(monoisopropylamino)-fluorophosphine oxide diluted to 0·05% (v/v) pure material plus 0·2% (v/v). Lissapol N.

(*i*) *Systox*. Commercial product (containing 50% (w/w) *O*, *O*-diethyl-*O*-ethyl-mercapto-ethyl thiophosphate) diluted to 0·05% (w/v) pure material plus 0·2% (v/v) Lissapol N.

METHODS

Spring beans were used in all experiments, Garton's in 1950 and 1951, Ashwell's in 1952. They were planted on 11 March 1950, 16 April 1951 and 18 March 1952.

For the field trials the beans were drilled in rows 20 in. apart and divided into randomized blocks, each comprising one unsprayed plot and one plot for each insecticide treatment. The designs of the experiments were as follows:

1950. Plot size 85 × 16 ft. = $\frac{1}{35}$ acre: two blocks of five plots each.

1951. Plot size 31 × 12 ft. = $\frac{1}{120}$ acre: four blocks of six plots each.

1952. Plot size 31 × 12 ft. = $\frac{1}{120}$ acre: two blocks of seven plots each. This experiment originally comprised four blocks in two pairs sited at opposite ends of a field. However, one pair of blocks was discarded owing to a very poor and patchy stand of beans. The plant stand in the remaining two blocks was also uneven.

The insecticides were applied by hand lance and a motor pump. Complete coverage of the upper halves of the bean stems was attempted, the volume of spray applied varying from about 220 to 270 gal./acre.

Field beans are closely planted and each may consist of more than one stem; separate plants are not easily distinguished and therefore all counts were made on a 'per stem' basis.

In the year 1950 peak aphid populations on each plot were estimated by measuring the length of aphid clusters on every infested stem (C. G. Johnson, unpublished). In 1951 and 1952 a different method (C. G. Johnson & J. A. Nelder, unpublished) was used and the populations were assessed at weekly intervals throughout the period of infestation. At each inspection twenty stems per row were examined at random; i.e. 140 stems per plot. The aphid population on each stem was put into one of the following categories (*a*) no aphids, (*b*) 1–10, (*c*) 10–100, (*d*) 100–500, (*e*) 500–1000, (*f*) 1000–10,000, (*g*) 10,000–50,000. The average numbers of aphids per stem were calculated from these data; the figures, although only approximations, gave useful information on the relative effectiveness of the different insecticides (see Figs. 1 and 2).

At each examination the active stages of certain aphid predators were counted. These were mainly Coccinellid adults and larvae with a few Syrphid and Chrysopid larvae.

When ripe, the crops from each plot were harvested separately and the weights of straw and seed obtained.

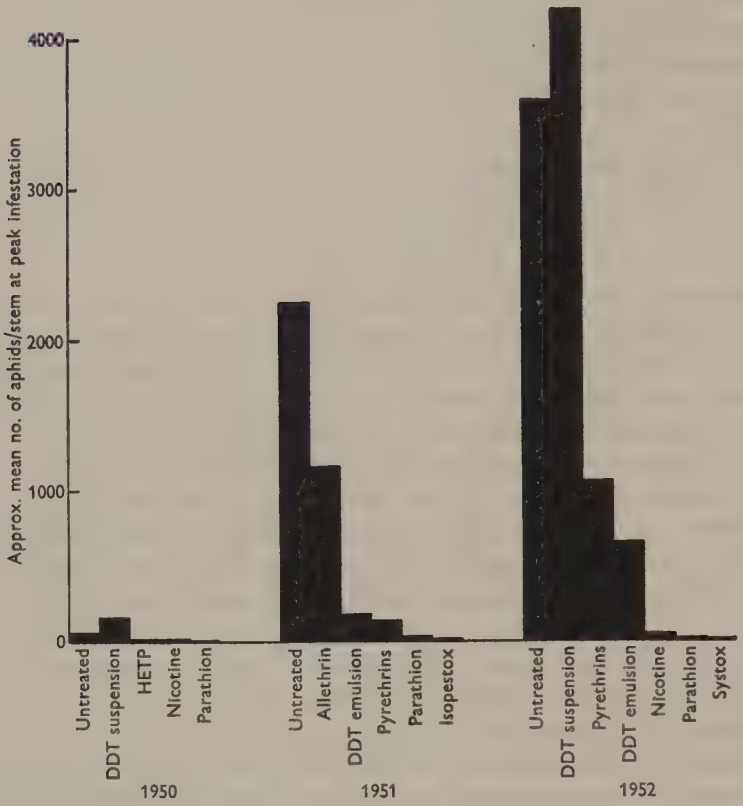

Fig. 1. Peak *A. fabae* populations in the years 1950, 1951 and 1952 on spring-bean plots treated with different insecticides.

Control in relation to life cycle of *Aphis fabae*

Alate primary migrants of *A. fabae* from the winter host usually reach the bean crop between mid-May and mid-June. They fly from plant to plant depositing batches of nymphs, which apparently are solely responsible for the big populations of apterae that often develop in late June and July. Few alatae are produced until mid-July when they become abundant. Although a few of these secondary migrants may colonize the beans and produce progeny, the plants at this stage are nearing maturity and appear unacceptable to the aphid.

Thus an effective insecticide treatment carried out just after the primary migration has ceased should, by eliminating the small initial infestation, stop *A. fabae* build-up and prevent damage throughout the rest of the growing season of the bean. In all the experiments this procedure was adopted, i.e. a single spraying was carried out as soon as it was estimated that primary migration had ceased. In practice no attempt was made to determine the precise end of primary migration.

Instead the beginning was determined and the spraying carried out about 4 weeks later, when it could be safely assumed that migration had ended. At the time of spraying, a few clusters of about 100 aphids were noticeable, but most were still hidden amongst the bracts surrounding the growing points of the stems.

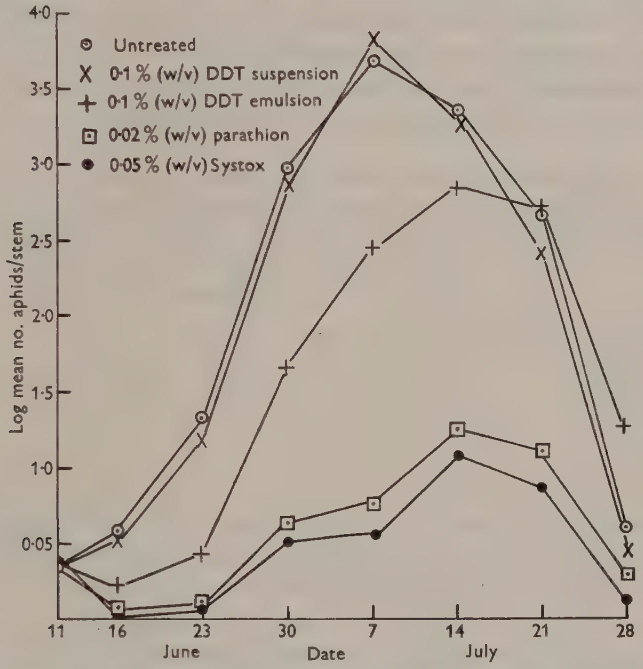

Fig. 2. Counts of *A. fabae* made in the year 1952 at intervals during an infestation on field beans treated with different insecticides.

Comparison of insecticides for control of *Aphis fabae* in the field

The insecticides were applied on 12–16 June 1950, 3 July 1951 and 12 June 1952. Fig. 1 gives the peak aphid populations per stem reached in each treatment; those on the untreated controls show that attack was slight in 1950 and severe in 1952. Details of the peak populations in the 1950 experiment are given in Table 1.

Table 1. *Approximate mean numbers of* Aphis fabae *per stem at the peak of infestation (5–13 July* 1950) *on spring beans treated with different insecticides*

Untreated control	0·1 % (w/v) DDT suspension	0·02 % (v/v) parathion	0·1 % (v/v) HETP	0·1 % (v/v) nicotine
59	166	3	6	8

It can be seen that parathion, HETP and nicotine reduced the aphid population while the DDT suspension caused a marked increase compared with the untreated control.

In 1951 the DDT suspension was not used; all treatments reduced the aphid populations, allethrin being the least and parathion and Isopestox the most effective (Fig. 1). An 0·1% DDT emulsion, unlike the suspension used in 1950, greatly reduced the aphid population.

In 1952, both of the DDT preparations were used and, as before, the suspension increased, and the emulsion decreased the aphid population. Parathion again gave efficient control as did Systox and nicotine (Fig. 1).

Counts of aphid populations in each treatment one day prior to spraying, and also at weekly intervals afterwards, showed the effect of early spraying.

TABLE 2. *Approximate mean numbers of aphids per stem before and after spraying field beans with different insecticides (year 1952)*

	One day before spraying	After spraying			
		4 days	11 days	18 days	25 days
Untreated control	2	4	21	955	3589
DDT suspension	2	4	15	832	4169
DDT emulsion	2	2	3	46	279
Pyrethrins	3	2	3	46	299
Nicotine	2	1	1	4	9
Parathion	2	1	1	4	6
Systox	3	0	1	3	4

Thus in 1952 (Table 2), one day before spraying, the mean number of aphids, consisting of primary migrants and their first progeny, was only 2–3 per stem. In the control and DDT-suspension-treated plots the aphids were not destroyed and, as shown in Fig. 2, their 'descendants' reached peak populations of 3589 and 4169 per stem respectively. In parathion and Systox treatments, where almost all aphids were destroyed, the populations reached peaks of only 16 and 12 aphids per stem respectively. These peaks and also those on the DDT-emulsion- nicotine- and pyrethrum-treated plots occurred 1–2 weeks after those on the control and DDT-suspension-treated plots (Fig. 2). This was due mainly to colonization by alate secondary migrants from the last-named plots and from surrounding untreated beans. These migrants were of little importance for they were produced when the crop was almost mature, and most migrated elsewhere. As shown in Fig. 2 the aphid population in the plots fell almost to zero during the following 2 weeks.

MODE OF ACTION OF INSECTICIDES ON *APHIS FABAE*

In the main field trials, each insecticide was used at only one concentration; more data on the relative effectiveness of the different materials were needed, and therefore additional laboratory and small-scale field experiments were carried out.

Contact toxicity of insecticides

Direct contact toxicity of the preparations used in the 1952 field trial was determined in the laboratory using the method described by Potter (1941, 1952*a*). Three replicates of fifteen adult apterous parthenogenetic females of *A. fabae* collected in the field were each sprayed (in 9 cm. Petri dishes with tricoline base), with a range of eight concentrations of each toxic ingredient, the media being identical with those used in the field trials. The volume of spray deposited per dish ranged from 560 to 581 mg. The aphids were kept in the sprayed dishes at a constant temperature of 20° C. and mortalities were recorded after 24 hr. The data were analysed by the method of probits (Finney, 1947), which showed that none of the curves was significantly heterogeneous. Table 3 gives the calculated LD 50's and LD 95's in g. toxic ingredient/100 c.c. of spray compared with the concentrations used in the field trial.

TABLE 3. *Results of a laboratory trial on direct contact toxicity of insecticides to adult apterae of* Aphis fabae, *showing the calculated LD* 50*'s and LD* 95*'s compared with the concentrations used in the field trials*

(Dose in g. toxic ingredient/100 c.c. spray)*

	DDT suspension	DDT emulsion	Nicotine	Pyrethrins	Systox	Parathion
LD 50	2·43	0·17	0·0081	0·00054	0·00040	0·00015
LD 95	—	13·4	0·022	0·00090	0·0010	0·00025
Concentration used in field trial	0·1	0·1	0·1	0·02	0·05	0·02
Ratio LD 95: field concentration	—	1:0·008	1:4·6	1:22	1:50	1:80

* Except nicotine which is given in c.c./100 c.c. spray.

The slopes of probit lines for both of the DDT preparations were relatively very shallow and thus it was calculated that high DDT concentrations were needed to give 95% kill of *A. fabae*; for the suspension the calculated LD 95 was impossibly high, and thus is not given in the Table. Being slow-acting, DDT would appear less toxic in the laboratory than in the field. However, even there, the suspension was ineffective and, although the emulsion gave fair control, its DDT content should be higher than 0·1% (w/v) for it to compare in efficiency with 0·1% (v/v) nicotine or 0·02% (w/v) parathion.

The field concentrations of the other insecticides all exceeded the figures for their LD 95's, and the extent to which this occurred, as shown in Table 3, suggests that the order of toxicity of the formulations should be parathion > Systox > pyrethrins > nicotine, and not Systox > parathion > nicotine > pyrethrins as was recorded in the field (Fig. 1). However, Systox by systemic action and nicotine by fumigant action may kill aphids not struck directly by the spray and thus be relatively more effective in the field where complete coverage of the crop is unlikely to be obtained. Also nicotine, unlike the other insecticides, is relatively harmless to Coccinellid predators of the aphid (Table 8).

Persistence of insecticides

This was studied in the field with the insecticide preparations at the concentrations used in the 1952 field trial. In June 1952, about 2 weeks before the plants had reached full height, plots of field beans 8 ft. square were sprayed thoroughly with each insecticide, the control being treated with the medium only. Immediately after spraying each bean stem was labelled at the youngest fully opened leaflet. Two hours later (when the foliage was dry), and also at daily intervals, five stems were each caged above the label in a bolting cloth 'sleeve'. Five *A. fabae* apterae were placed in each cage, a total of 25 aphids per treatment. 7–8 days later the cages were opened and the aphids carefully counted. The results are given in Table 4.

TABLE 4. *Persistence of toxicity to* Aphis fabae *of insecticide spray residues on young stems of sprayed field beans*

(Number of live aphids produced after 7–8 days.)

Interval after spraying before introducing 25 adult apterae	Medium-treated control	0·1 % (w/v) DDT suspension	0·1 % (w/v) DDT emulsion	0·1 % (v/v) nicotine	0·02 % (w/v) pyrethrins	0·02 % (w/v) parathion	0·05 % (w/v) Systox
2 hr.	533	313	78	449	321	0	0
24 hr.	857	360	290	726	681	103	32
2 days	—	—	—	—	602	261	94
3 days	744	—	—	—	—	722	122
4 days	618	—	—	—	—	728	56
5 days	725	—	—	—	—	—	253

The table shows that only the parathion and Systox preparations caused 100% kill of aphids caged on the treated bean plants 2 hr. after spraying. By this time the nicotine and pyrethrins spray films were ineffective; successful reproduction also occurred on the DDT treatments. The parathion-treated stems were noticeably but not completely toxic for 2 days, after which the aphids reproduced on them as rapidly as on the controls. Loss of persistence was not due to dilution of the film by plant growth because on the same treated plot the spray residue on full-grown leaves had also lost all toxicity after 2 days. Table 4 shows that the Systox-treated plants remained toxic for 5 days after treatment, though like parathion they ceased to cause 100% kill within 24 hr.

Effect of *Aphis fabae* control by different insecticides on bean yield

Fig. 3 gives, for each treatment of the three field trials, the yields per acre of seed and stems in relation to mean number of aphids per stem at the peak infestation.

In 1950, aphid attack was slight and the insignificant differences between yields of seed from parathion-, HEPT-, and nicotine-treated plots compared with the untreated controls suggests that the peak aphid population of 59 per stem on the control was of no economic importance. It is interesting that the lower yield from plots treated with the DDT suspension is correlated with a higher aphid population, though the yield (10·7 cwt./acre) is not significantly different from the control yield of 11·9 cwt./acre (S.E. of mean = ± 1·08).

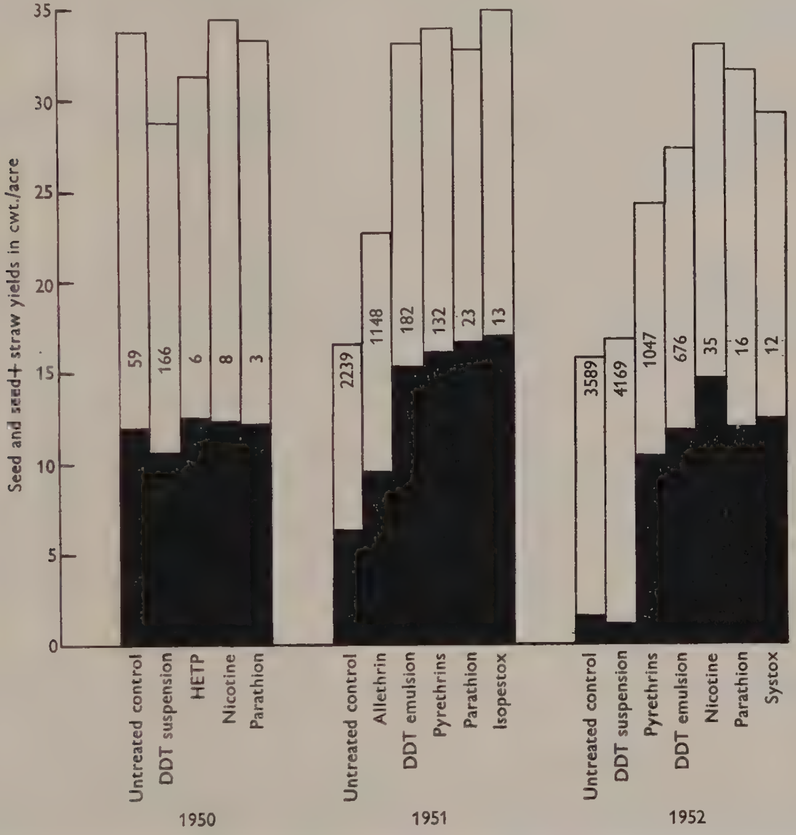

Fig. 3. Yields of seed (solid rectangles) and seed + straw (open rectangles) in the years 1950, 1951 and 1952 obtained from spring-bean plots treated with different insecticides for *A. fabae* control. The approximate numbers of *A. fabae* per stem at peak infestation are shown for each treatment.

In 1951 there was a marked correlation between aphid population and seed yield; where aphid control was most efficient (parathion and Isopestox treatments) the yield was the highest, being about × 2·7 that of the control. All treatments gave significantly higher seed yields (9·6–17·1 cwt./acre) than the control (6·3 cwt./acre; S.E. of mean = ± 0·71); also the yields from DDT emulsion, pyrethrum, parathion, and Isopestox treatments (15·2–17·1 cwt./acre) were significantly higher than that of the allethrin treatment (9·6 cwt./acre).

In 1952, due to heavy aphid attack, the seed yields of the untreated control and of the DDT-suspension-treated plots were only 1·4 and 1·3 cwt./acre respectively

—less than the amounts sown. All other treatments give moderate to excellent aphid control and significantly higher seed yields of 10·3–14·8 cwt./acre (S.E. of mean = ± 3·19). Differences between seed yields of pyrethrins, DDT emulsion, nicotine, parathion and Systox treatments were not significant in spite of differences in effectiveness of aphid control. However, no importance can be attached to this because the plant stand was patchy.

It is interesting that the straw yields (Fig. 3), unlike the seed yields, were only slightly reduced by *A. fabae* attack which, it seems, has relatively little effect on the vegetative development of the bean plant.

NATURE OF *APHIS FABAE* DAMAGE TO FIELD BEANS

In 1952, the growth of aphid-infested and aphid-free field-bean plants was compared; 42 stems were labelled at random in two untreated control plots of the field trial and their development compared with 42 similarly chosen from two parathion-treated plots. The peak aphid populations averaged 3957 per stem on the untreated and 2·5 on the parathion-treated stems; thus, on the latter, aphid damage was probably insignificant.

The labelled stems were examined at intervals until harvested; the counts of flowers and pods produced are given in Table 5.

TABLE 5. *The effect of* Aphis fabae *attack on field beans: mean numbers of flower trusses, flowers and mature pods, and weights of seed produced per stem by untreated and parathion-treated plants*

Treatment	Flower trusses	Flowers	Flowers set	Ripe pods	Seed yield (g.)
Untreated	9·7	42·5	11·3	1·6	0·80
Parathion	9·0	47·4	13·3	4·7	6·78

It can be seen that aphid attack had little effect on flower formation or on the number of flowers set, probably because at this stage in growth of the plant the aphid population was still relatively low. However, in July the aphids became abundant on the untreated plots and caused almost all developing pods to drop or shrivel before reaching maturity. It is noteworthy that in the parathion-treated plots, although aphid attack was insignificant, a mean total of 47·4 flowers per stem produced only 4·7 ripe pods. The latter were all borne on the first four trusses; many flowers set on the later trusses, but the pods dropped at an early stage of development. Probably the dry summer was partly responsible, though normally only 10–20% of field-bean flowers give ripe pods (Soper, 1952).

The seeds from the labelled stems were harvested and divided into normal (smooth, rounded and usually over 0·1 g. in weight) and abnormal (wrinkled, often misshapen and less than 0·1 g. in weight); details are given in Table 6.

It can be seen that aphid attack not only reduced the yield but also the quality of the seed. This was confirmed by the seed sizes of samples taken from the total crop harvested in each treatment of the 1952 field trial (Table 7).

TABLE 6. *Yield and quality of seed from untreated and parathion-treated field beans*

	Total no. of seeds per stem		Mean wt. of each seed (g.)		% viable seed	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Untreated control	2·3	4·1	0·32	0·029	97·4	57·7
Parathion	12·2	4·3	0·56	0·007	99·5	20·1

TABLE 7. *Size of spring-bean seed from plots treated with different insecticides to control* Aphis fabae

	Untreated control	0·1% (w/v) DDT suspension	0·1% (w/v) DDT emulsion	0·02% (w/v) pyrethrins	0·1% (v/v) nicotine	0·02% (w/v) parathion	0·05% (w/v) Systox
Mean wt. of each seed (g.)	0·39	0·27	0·54	0·49	0·64	0·62	0·65

Plots treated with the DDT suspension bore the highest aphid population (Fig. 1) and produced the smallest seeds. Bigger seed came from the plots treated with pyrethrins and the DDT emulsion, which gave partial aphid control, while the largest seeds came from those treated with the most efficient aphicides—the nicotine, parathion and Systox sprays.

EFFECT OF INSECTICIDES ON PREDATORS OF *APHIS FABAE*

The active stages of some *A. fabae* predators were counted at intervals in all plots of the 1951 and 1952 field trials. Over 95% were adult and larval Coccinellidae (mainly *Coccinella septempunctata* and *Adalia bipunctata*), the remainder being Syrphid and Chrysopid larvae. A few Anthocoridae and Cecidomyiidae were seen but were not counted. *A. fabae* parasitism was not studied in 1951, while in 1952 parasitized aphids were seen only towards the end of the infestation. Thus Coccinellidae appeared to be the most important natural enemies of *A. fabae*.

The laboratory technique described by Potter (1941, 1952*a*) was used to compare the contact toxicity to Coccinellidae of the insecticide preparations used in the 1952 field trial. Three replicates of ten adult *Coccinella septempunctata* and of ten adult *Adalia bipunctata* collected in the field were sprayed with each insecticide at its field concentration. The insects were kept in the sprayed Petri dishes at 20° C. and the kills recorded after two days.

Results in Table 8 indicate that *Coccinella septempunctata* is more resistant than *Adalia bipunctata*. As shown previously (Ripper, 1944; Way, 1949) nicotine had little or no effect on either species; parathion, Systox and the pyrethrins caused high kills of both species whilst the DDT suspension was notably toxic to *A. bipunctata*. In a further experiment, the DDT suspension as a dry residual film on the inside of Petri dishes caused 90·0%, and the DDT emulsion, 16·7% kill of batches of 30 adult *A. bipunctata*.

In 1950 and 1952, the relatively high aphid populations which developed in plots treated with 0·1% (w/v) DDT suspension (Fig. 1) were probably the result of its insignificant effect on *A. fabae* (Table 3) and its notable toxicity to Coccinellid predators (Table 8).

TABLE 8. *Percentage kills of two species of adult Coccinellidae sprayed with various insecticides in the laboratory*

Treatment	*C. septempunctata*	*A. bipunctata*
Medium only*	0	3
0·1% (v/v) nicotine	0	13
0·02% (w/v) pyrethrins + 0·2% (v/v) piperonyl butoxide	96	100
0·1% (w/v) DDT suspension	8	90
0·1% (w/v) DDT emulsion	0	48
0·02% (w/v) parathion	100	100
0·05% (w/v) Systox	100	100

* Consisting of 0·2% (v/v) Lissapol N and 0·4% (v/v) 50:50 benzene, toluene in water.

SIGNIFICANCE OF *APHIS FABAE* PREDATORS

Table 9 shows, for some treatments of the 1952 field trial, the ratios between the numbers of active predators and the approximate numbers of the *A. fabae* host at intervals during the period of aphid attack.

Clausen (1916) has shown that last instar larvae and adults of average size Coccinellidae may eat as many as 50 aphids per day. Thus in 1952 at the beginning of aphid infestation (Table 9, '1 day before spraying') when the predator aphid ratio varied from 1:58 to 1:310 it is probable that predator attack was significantly affecting the aphid population. However, as shown by counts on the untreated controls (Table 2) the aphid population increased rapidly regardless of predation and after 25 days the predator aphid ratio 1:41,700 showed that at this stage natural enemies were of insignificant importance.

The nicotine, parathion and Systox preparations were good aphicides (Fig. 1); also the two last-named insecticides, unlike nicotine, probably killed all Coccinellid adults present at the time of application (Table 8). However, by reducing the initial aphid population almost to zero they gave rather better control than nicotine (Fig. 1 and Table 2), despite their toxicity to Coccinellidae. It is interesting that 11–25 days after spraying, when a few aphid clusters reappeared on the parathion and Systox-treated plots, Coccinellidae also reappeared. Compared with pest density (4–4169 per stem), predator density (0·021–0·086 per stem) at 25 days was relatively uniform over the whole experimental area. Thus at this time, predators on the Systox-treated plots (predator aphid ratio = 1:120) were probably influencing the aphid population whereas on the untreated and DDT-suspension-treated plots (predator, aphid ratios = 1:41,700 and 1:115,800 respectively), their effect was undoubtedly negligible.

Table 9. *Relationship between number of active predators and numbers of* Aphis fabae *in plots treated with different insecticides*

			After spraying			
	1 day before spraying		4 days		11 days	
	Predators/stem	Ratio, no. of predators:no. of aphids	Predators/stem	Ratio, no. of predators:no. of aphids	Predators/stem	Ratio, no. of predators:no. of aphids
Untreated control	0·0071	1:310	0·011	1:345	0·0071	1:3010
DDT suspension	0·0071	1:310	0·0036	1:1050	0·0071	1:2040
DDT emulsion	0·025	1:92	0·0036	1:440	0·0036	1:720
Nicotine	0·036	1:58	0·014	1:93	0·0036	1:390
Parathion	0·025	1:88	0	—	0	—
Systox	0·014	1:190	0	—	0	—

	After spraying			
	25 days		39 days	
	Predators/stem	Ratio, no. of predators:no. of aphids	Predators/stem	Ratio, no. of predators:no. of aphids
Untreated control	0·086	1:41,700	0·27	1:1730
DDT suspension	0·036	1:115,800	0·20	1:1250
DDT emulsion	0·057	1:4,880	0·34	1:1,440
Nicotine	0·054	1:160	0·24	1:140
Parathion	0·021	1. 280	0·24	1:55
Systox	0·029	1:120	0·16	1:46

Discussion

This work has shown that a single early spraying with any one of several insecticides may, by controlling *A. fabae*, greatly increase the yield of spring bean seed. For example, treated plots gave increased yields of about × 2·7 in the year 1951 and of about × 7–10 in 1952. However, it is realized that much remains to be done both on *A. fabae* damage assessment and on methods of control. The severity of *A. fabae* attack may vary greatly from year to year (see Fig. 1) and it is not known how often the increased yields would repay successful control. Such data are required before early spraying can be recommended, because this is done at a time when economic loss by *A. fabae* cannot be predicted with certainty. Thus, even if most bean plants are infected by the primary migrants it is always possible that adverse weather will curtail what promises to be a severe attack. This disadvantage of early spraying must be weighed against its advantages; for example, it stops all but negligible *A. fabae* damage compared with spraying later, when the aphid may already have caused much damage.

Spring beans generally yield less seed than those sown in autumn; they also appear more susceptible to *A. fabae* damage. Thus the spring bean crop may fail owing to aphid attack while autumn beans, if not seriously damaged by a severe winter or by chocolate spot disease generally give an economic return. However, no data are available on crop loss of autumn beans due to *A. fabae* attack which although not catastrophic may often justify control measures.

It has been shown that some insecticides by destroying beneficial insects may greatly increase plant damage by certain pest species (Steiner, Arnold & Summerland, 1944; Hough, Clancy & Pollard, 1945; Collyer, 1949). It is significant that this problem has arisen with perennial tree crops. These form a relatively stable environment for insects, many of whom overwinter on the plant, and during its long growing season attain equilibrium, providing the process is not upset by insecticides.

By contrast, the environment of annual crops such as the field bean is relatively very unstable. Except in the soil, a fresh insect community develops annually on each crop, and it seems unlikely that any equilibrium is established between pest or potential pest insects and their natural enemies, especially as the growing season of the plant, and hence the period during which insects can multiply on it, is relatively short. This suggests that beneficial insects have less effect on pests of annual crops than on those of perennial tree crops. Hence, insecticides which destroy them are less likely to cause dangerous increases in pest populations. Nevertheless, as shown by this work, *A. fabae* may noticeably benefit from destruction of its Coccinellid predators; for example, 0·1 % (v/v) nicotine was harmless to Coccinellids and gave rather better field control of *A. fabae* than 0·02 % (w/v) natural pyrethrins which, although a better aphicide, was toxic to the Coccinellids. Also, an 0·1 % (w/v) DDT suspension increased the aphid population by selectively destroying many Coccinellidae. However, Table 9 suggests that predators and parasites of *A. fabae* are unlikely to be economically important unless it happens that relatively large numbers migrate into the crop at the beginning of aphid infestation when the pest population is relatively low. At this time, and under these conditions, an insecticide which selectively destroys the predators may cause harm by increasing the *A. fabae* population, but it seems that none will be caused by an insecticide such as 0·01 % (w/v) parathion or 0·05 % (w/v). Systox which, although highly toxic to its predators, efficiently controls *A. fabae* (Fig. 1 and Table 8).

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THE CONTROL OF MANGOLD FLY (*PEGOMYIA BETAE* CURTIS) WITH DDT AND OTHER CHLORINATED HYDROCARBONS

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(With 4 Text-figures)

Laboratory and field trials were carried out in the period 1948–52 with DDT, BHC, and other chlorinated hydrocarbon insecticides and with parathion for the control of mangold-fly larvae (*Pegomyia betae* Curtis) mining in the leaves of sugar beet. Kills ranging from 85 to 99% were obtained within 6 days with 'high'- and 'low-volume' sprays using 6–24 oz. of DDT per acre in the form of an oil emulsion applied as a top cover only. Formulation in oil improved the action of the DDT, it being necessary to use dusts and wettable powders at excessive rates to obtain equivalent kills. DDT was usually superior to BHC when compared at customary rates of application for both insecticides. When oil emulsions were compared side by side, however, as 'low-volume' sprays, DDT was not significantly better than BHC. In the same trial, toxaphene, aldrin and dieldrin also gave kills of the same order as BHC and DDT. With the possible exception of parathion, none of the insecticides used appeared to have any effect upon eggs.

Plot yields of two of the trials were determined in the autumn, but only at one trial was there a significant increase in yield on the treated plots. At this trial the mangold-fly infestation was severe, there being twenty-five unhatched eggs and twenty larvae per plant at the time of treatment. Attempts were made to assess the degree of defoliation and to relate it to the larval population and to the effect on yield.

Commercial insecticides of the 'stock emulsion' and 'miscible oil' types occasionally caused phytotoxicity when applied to sugar beet at excessive, 'high-volume' rates and concentrations. More serious phytotoxicity was observed in tests of commercial insecticides used at 'low-volume' rates and concentrations. Out of ten products tested, only three were acceptable over the whole range of rates and concentrations employed, whilst four caused injury over the whole range.

The results obtained in the field and the laboratory were in agreement but did not always agree with the findings of other workers. BHC has previously been recommended largely on the basis of experiments on larvae mining very small beet plants when in, or just beyond, the cotyledon stage. All the trials by the authors were on sugar-beet seedlings with about six foliage leaves and normal in size for the time of the year. On these plants BHC proved ineffective both as dust and 'high-volume' spray when used at customary rates, but was effective as a 'low-volume' spray in oil emulsion.

The successful control of mining larvae by DDT and other chlorinated hydrocarbons is probably due to penetration of the leaf tissue by the insecticide.

Before the introduction of modern synthetic insecticides, there was no satisfactory method of controlling attacks on sugar beet or mangold by the mangold fly, *Pegomyia betae* Curtis (syn. *P. hyoscyami* var. *betae* Curtis). The use of poison-bait spray against the flies, introduced by workers in Germany in 1926 and frequently employed on the Continent, has been reviewed by Roebuck, Baker & White (1945). The success of this method depends too much on concerted action in beet-growing areas and on the careful timing of a number of applications. Moreover, mangold-fly attacks are sporadic, especially in Great Britain, and routine preventive treatment with poison-bait sprays, or with modern insecticides, would prove unnecessary and uneconomical in most years, even in the areas where the pest is most prevalent. For these reasons, control measures are best directed against the freshly hatched larvae in the mines when they appear sufficiently numerous to cause serious injury.

The concealed habit of the larva renders it difficult to kill with the older types of insecticide. Although effective kills were sometimes observed incidentally when nicotine fumigation was being used against the bean aphid (*Aphis fabae* Scop.) on sugar beet, this method is too costly and too cumbersome for general use. The following laboratory experiments and field trials made in the period 1948–52 show that DDT in oil emulsion is capable of giving highly effective kills. Excellent kills may also be obtained by 'low-volume' spraying with BHC, toxaphene, aldrin or dieldrin in oil emulsion.

LABORATORY EXPERIMENTS

The effect of a number of proprietary insecticides upon mangold-fly eggs and larvae in various stages of growth was tested in the laboratory, using an apparatus built by D. W. Wright and G. A. Wheatley of the National Vegetable Research Station with which it was possible to apply approximately constant amounts of insecticidal dust or spray to plants.

Cultures of mangold flies were maintained in cages and the females readily laid eggs upon sugar-beet plants placed therein. After suitable intervals, depending on whether eggs or larvae were to be treated, leaves were removed from the plants and the petioles placed in test-tubes of water, the necks being plugged with cotton-wool. The leaves were randomized among the treatments, so that there were equal numbers of leaves and approximately equal numbers of eggs or larvae for each treatment. The leaves were sprayed or dusted from a distance of 80 cm. for 20 sec. whilst rotating five times on a turn-table. Constant charges of insecticide were utilized throughout the experiments, and in this way a fine even cover was obtained on both sides of the leaves. Treated leaves were kept in racks at room temperature and counts of eggs, living and dead larvae, were made at intervals. Sometimes observation of results was marred by yellowing and withering of leaves or by the migration and loss of some of the larvae. Larvae thus lost had to be recorded as living, although, in fact, many may have died after contact with the film of insecticide on the leaf surface. This migration was most noticeable when large larvae were being treated, and experiments with these were unsuccessful.

Experiment 1. Treatment of eggs

In this experiment there were six treatments and three untreated controls, each comprising five leaves and about fifty eggs. Details of the treatments applied and the results obtained are set out in Table 1.

TABLE 1. *The effects of various insecticides upon mangold fly eggs in the laboratory*

	Results[*]					
	After 3 days			After 4 days		
	Unhatched eggs (%)	Small larvae (%)		Unhatched eggs (%)	Small larvae (%)	
Treatments		Alive	Dead		Alive	Dead
Calomel dust, 4 %[†]	21	79	0	15	56	29
BHC dust, 2·5 % (0·33 % γ)	11	0	89	11	0	89
DDT-oil emulsion, 1 %	48	35	17	46	0	54
DDT-oil emulsion, 0·25 %	67	0	33	62	0	38
DDT dust, 2 %	33	21	46	29	2	69
DDT dust, 5 %	26	6	68	19	4	77
Controls (untreated) A	32	68	0	32	60	8
B	41	59	0	39	49	12
C	76	24	0	76	22	2

[*] The total number of eggs for each treatment was approximately fifty and the figures given are percentages of these totals.

[†] Three leaves bearing only thirty-four eggs, the other two leaves being disregarded because of severe wilting.

None of the treatments appeared to give a kill of eggs, but all treatments, except 4% calomel dust, gave a high percentage kill of larvae. It was observed here, and subsequently, that larvae appeared to hatch from eggs after treatment, and were killed only on entering the leaf. The low and variable percentage hatch of the eggs shown by treatments and controls was probably due to the somewhat unnatural conditions under which the flies were kept, i.e. in cages in the insectary.

Experiment 2. Treatment of young larvae in mines

In this experiment there were also six treatments and three untreated controls, each comprising five leaves and about eighteen young larvae. The treatments applied, and the results achieved, are shown graphically in Fig. 1. The 5% DDT dust with oil was prepared from the ordinary dust by the incorporation of 2% by weight of methyl naphthalene. This dust adhered more readily to the leaves than the ordinary 2% and 5% dusts.

An excellent kill of larvae was given by the DDT-oil emulsions and a reasonable kill by the dusts, especially the one containing oil.

Experiment 3. *Comparison of DDT emulsion and parathion*

Parathion spray at 0·01 % was compared with 0·125 % DDT-oil emulsion and an untreated control, employing about seventy young larvae in each case. The results are shown in Table 2. The parathion killed the larvae in the mines far more

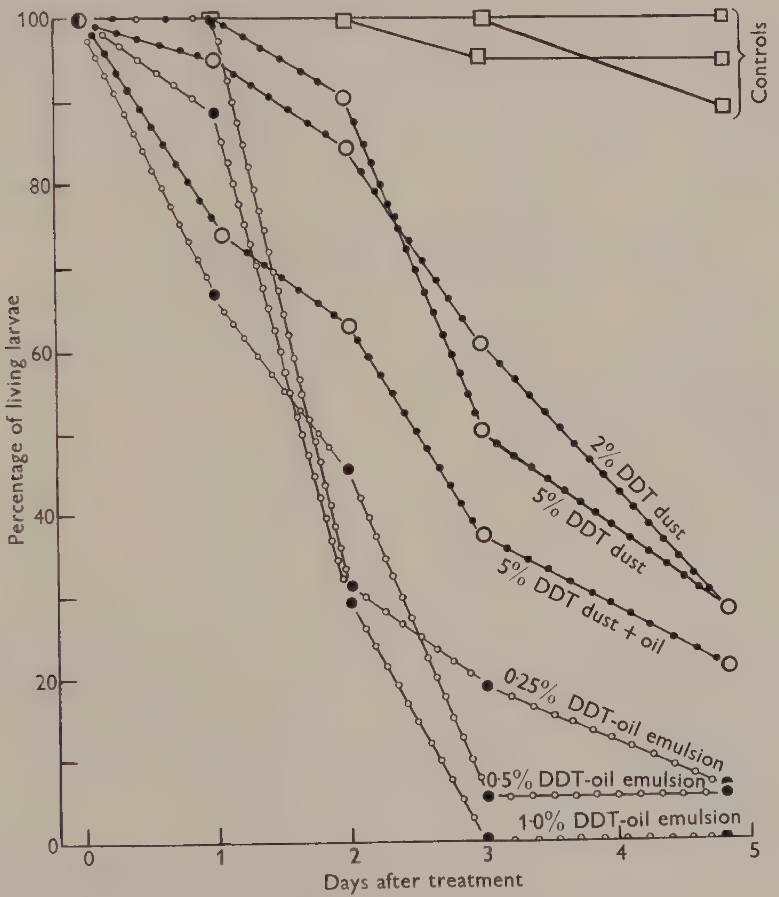

Fig. 1. Control in the laboratory of young larvae in mines, using DDT.

Table 2. *Comparison of the effect of parathion and DDT-oil emulsion on young larvae in mines*

Treatment	Number of larvae alive after			
	0 hr.	18 hr.	24 hr.	40 hr.
Parathion, 0·01 %	69	1	0	—
DDT-oil emulsion, 0·125 %	69	21	2	0
Control	71	68	67	63

rapidly than did the DDT-oil emulsion, but even the DDT treatment had produced 100% kill within 40 hr. of application.

Experiment 4. Comparison of solvent naphtha emulsion with and without DDT

The stock DDT-oil emulsion used in all the laboratory experiments consisted of 16·7% DDT, 55% solvent naphtha, and a small percentage of a proprietary wetting agent. Equivalent strengths of emulsion (0·41% solvent naphtha), with and without DDT, were compared with an untreated control, using six leaves and nineteen larvae in each case. Despite the small numbers of larvae employed, the results set out in Table 3 show clearly that the emulsion alone was not toxic to them.

TABLE 3. *Comparison of the effect of oil emulsion with and without DDT on young larvae in mines*

Treatment	Number of larvae alive after					
	0 hr.	8 hr.	24 hr.	45 hr.	57 hr.	69 hr.
Oil emulsion + DDT (0·41% solvent naphtha + 0·125% DDT)	19	19	15	7	3	1
Oil emulsion alone (0·41% solvent naphtha)	19	19	19	17	17	17
Control	19	19	18	18	16	16

FIELD TRIALS

Field trial no. 1

A preliminary field trial was done in 1948 at Ramsey, Hunts, to determine the effects of DDT and BHC dusts and sprays on a low infestation of larvae mining in the leaves. Details are given in Table 4. Sprays were applied with a knapsack sprayer, giving a heavy top and bottom cover. Dusts were applied by 'hand-puffers', each plant being treated individually and given a complete top and bottom cover.

The effect of the insecticides was determined 15 days after treatment by counting the number of living larvae in a sample of leaves from each plot. The samples, consisting of twenty-five leaves each, were obtained by removing all mined leaves from plants taken at random in each plot, senescent cotyledons being neglected. The rates of application of the insecticides and their effects are set out in Table 5.

A trial on this scale, without replication, gives no more than an indication of the relative value of different treatments. Nevertheless, it appears that 0·5 and 1% DDT wettable powder, 0·25, 0·5 and 1% DDT-oil emulsion, and 0·04% γ-BHC suspension gave a fair measure of control of larvae mining in the leaves within 15 days of treatment. All other insecticides gave only a partial control.

TABLE 4. *Details of the various trial sites*

	Trial 1	Trial 2	Trial 4
Year	1948	1949	1950
Locality	Ramsey, Hunts	Massingham, Norfolk	Burgh St Peter, Lowestoft
Soil type	Black fen	Sandy loam with flints	Sandy loam
Date of sowing	End of March	End of March	9 March
Date of singling	Early May	5 May	Early May
Date of treatment	20 May	20 May	29 May
Plant size at time of treatment: no. of foliage leaves	6	6	6
Maximum infestation:			
Unhatched eggs/plant	4·2	24·8	6·0
Larvae/plant	1·6	22·9	9·0
Plot size	11 yd. × 8 rows $\frac{1}{110}$ acre	22 yd. × 8 rows $\frac{1}{41}$ acre	22 yd. × 8 rows $\frac{1}{55}$ acre
Layout	14 treatments each with local controls, 28 plots	13 treatments, 3 controls, triple replication, 48 plots	4 treatments, 1 control, 5 × 5 Latin square, 25 plots
Sampling unit per plot	25 mined leaves	30 mined leaves	36 leaves

	Trial 5	Trial 6	Trial 7
Year	1950	1952	1952
Locality	Corton, Lowestoft	Norton, Loddon	Gorleston, Yarmouth
Soil type	Medium loam	Sandy loam	Sandy loam
Date of sowing	18 March	18 April	Mid-April
Date of singling	23 May	10 May	Mid-May
Date of treatment	29 May	21 May	29 May
Plant size at time of treatment: no. of foliage leaves	6	4	4
Maximum infestation:			
Unhatched eggs/plant	10·0	4·0	4·7
Larvae/plant	14·0	4·9	7·2
Plot size	22 yd. × 8 rows $\frac{1}{52}$ acre	80 yd. × 5 rows $\frac{1}{23}$ acre	100 yd. × 5 rows $\frac{1}{18}$ acre
Layout	4 treatments, 1 control, 5 × 5 Latin square, 25 plots	7 treatments, 1 control, 8 × 4 randomized block, 32 plots	6 treatments, 1 control, 7 × 4 randomized block, 28 plots
Sampling unit per plot	36 leaves	40 leaves	60 leaves

Field trial no. 2

A severe first-generation attack at Massingham, Norfolk, in 1949, provided an opportunity for a full-scale field trial, using the experience gained in 1948, and details of the trial site are given in Table 4. Various dusts, wettable powders and oil emulsions were used. Sprays were applied with a wheel-barrow-type, engine-driven sprayer fitted with 'high-volume' nozzles working at 60 lb./sq.in. Four rows were treated at a time and a good top cover was given to the plants. The nozzles were set directly over the rows and gave cones of spray 6 in. in diameter at ground-level. Thus the rate of application, 68 gal./acre, refers to treatment of

TABLE 5. *Field trial 1. Ramsey, Hunts, 1948: results of treatment with various insecticides*

Treatment	Concentration (%)	Rate of application per acre	Wt/acre (oz.)	Living larvae/25 mined leaves 15 days after treatment	
				Treated	Control
DDT					
Dust	2	110 lb.	35	40	32
	5	165 lb.	132	16	22
Wettable powder	0·25	110 gal.	44	14	44
	0·5		88	2	29
	1		176	1	50
Oil emulsion	0·25		44	2	29
	0·5*		88	0	41
	1*		176	0	33
BHC					
Dust 2 %	0·26γ	50 lb.	2†	43	20
2·5 %	0·33γ	60 lb.	3	27	31
3·75 %	0·49γ	55 lb.	4	44	40
Suspension	0·01γ	110 gal.	2	28	32
	0·02γ		4	25	27
	0·04γ		7	4	34

* The 1 and 0·5 % DDT-oil emulsion caused appreciable scorching of the foliage.

† Here and elsewhere the wt./acre is in terms of γ-BHC.

the rows only. Dusts were put on by hand with small 'puffers' at the rate of 44 lb./acre, each plant being treated individually. The application of sprays was similar to commercial practice, although the gallonage per acre was high, but the hand application of the dusts was far more thorough than could be obtained by any commercial method.

The plots were first sampled on 25 May, 5 days after treatment. All the mined leaves were picked from ten plants per plot, the plants being taken at random. The samples were kept in separate paper bags and held overnight at 42·5° F. to prevent further feeding of the living larvae, while the samples were waiting for the living and dead larvae to be counted. The results of these samples were eventually discarded on the grounds that they were derived from an inadequate number of sampling points, and a further sample of one mined leaf from each of thirty plants per plot was taken on the tenth day after treatment. Although the results of the first sample have been neglected in the detailed analysis below, they agreed closely with those of the second sample.

The degree of control obtained from the various treatments was measured by counting the living and dead larvae in the samples of mined leaves. After effective treatments, living larvae were few in number and did not prevent the accurate counting of dead larvae in the mines. When treatments were ineffective and when the control plot samples were being counted, the large numbers of living larvae had mined the leaves extensively, preventing accurate counting of the smaller, dead larvae. Hence, it was concluded that the numbers of living larvae give a better assessment of the effects of different treatments, provided that the samples are taken before the larvae mature and leave the leaves to pupate in the soil, i.e. within approximately 15 days of the eggs hatching. The same difficulty in the accurate counting of dead larvae was encountered in some subsequent trials, but the initial infestations were lower and the difficulty less serious.

The plots were lifted in late September to obtain the yields of roots and sugar. Plant populations were also counted for correction of the yields by analysis of covariance. Details of the treatments applied and of their effects on the larval population and on yield are set out in Table 6.

DDT preparations were superior to those containing BHC, and oil emulsions containing 0·4, 0·2 and 0·1% DDT were highly effective. Comparable performances were given by the highest strength of wettable DDT (1% DDT) and by the ordinary 5% DDT dust. The BHC suspension gave poor results, even at a concentration of 0·04% γ-isomer which is about four times that normally recommended by the manufacturers, and BHC dusts gave only a moderate performance. Both DDT and BHC dusts would probably be ineffective when applied by less thorough commercial methods. The plots treated with DDT-oil emulsions were outstanding and could be readily distinguished from the side of the field; the plants on these plots grew away vigorously and were inches higher than those on plots receiving other treatments. The plot-yields, however, failed to show this difference, so apparent immediately after treatment, and there was no correlation between yield and kill of larvae. Only the DDT dusts gave increases in the yield of washed beet that were significantly higher than the controls and some of the other treatments. Differences in sugar percentage were insignificant, although the dust treatments, taken as a group, gave increases approaching significance. In the yields of sugar, the DDT dusts again gave values significantly higher than the controls and some of the other treatments, as also did the weaker BHC dust. Evidently some disturbing factor overrode the early benefits obtained from mangold-fly control. The possibility of a partial control of the primary infestations of the bean aphid by the residual toxicity of the insecticides was foreseen, but counts made in June revealed no obvious differences in aphid populations. Another possibility was the partial control of *Myzus persicae* Sulz. and, through it, of virus yellows, particularly by the heavy and careful application of dusts. The DDT sprays that gave good control of mangold fly increased the value of the crop over the controls by from £2 to £4. 10*s*. per acre, and this sum represents the best estimate of the financial loss incurred from the pest that can be obtained from this particular trial.

Field trial no. 3

A small-scale subsidiary trial against the second generation attack at Massingham in 1949 was designed to compare parathion with DDT-oil emulsion. A 3 × 3 Latin square with individual plots measuring 10 × 4 yd. was used. The attack was at

TABLE 6. *Summary of treatments and results of field trial 2, Massingham, Norfolk,* 1949

Treatments	No.	Concentration of the insecticide (%)	Rate of application	Wt./acre (oz.)	Living larvae per 30 leaves, mean of 3 replicates: Actual	Living larvae per 30 leaves, mean of 3 replicates: √Actual	% kill	Yield of washed beet, tons per acre (corrected for plant population)	% sugar	Cash value per acre (estimate) £
Sprays										
DDT-oil emulsion	(1)	0·4	68 gal./acre applied mainly to the rows	44	2·0	1·1	99	11·21	14·73	56·65
	(2)	0·2		22	2·0	1·4	99	11·20	14·43	55·75
	(3)	0·1		11	16·0	4·0	92	10·93	15·03	56·05
DDT wettable powder	(4)	1·0		109	4·7	2·0	97	11·21	15·00	57·39
	(5)	0·5		55	35·7	7·6	81	10·75	15·03	55·13
	(6)	0·25		27	59·3	5·9	69	10·31	14·80	52·29
BHC suspension	(7)	0·04γ		4	136·7	11·5	28	10·70	14·73	54·07
	(8)	0·02γ		2	160·7	12·6	15	11·21	14·87	57·02
	(9)	0·01γ		1	158·0	12·5	17	10·87	14·30	53·75
Dusts										
DDT dust	(10)	5	44 lb./acre, applied to plants only	35	12·7	3·3	93	12·11	15·00	62·00
DDT dust+oil	(11)	5+oil		35	22·0	4·6	88	12·33	15·13	63·54
BHC dust	(12)	2·5 (0·33γ)		2	30·0	5·1	84	11·27	15·23	58·36
	(13)	3·75 (0·49γ)		3	45·0	6·6	76	11·34	15·13	58·44
Controls	(14–16)	No insecticide	—	—	190·2	13·7	0	10·48	14·72	52·93
Means	—	—	—	—	78·5	7·5	—	11·06	14·85	56·81
Standard errors										
Treatments 1–13	—	—	—	—	—	±0·7	—	±0·417	±0·28	—
Controls	—	—	—	—	—	±0·4	—	±0·241	±0·16	—
1–13/controls	—	—	—	—	—	±0·8	—	±0·482	±0·32	—
Variance ratios										
Treatment/error 13 to 32 D.F.			—	—	—	51·01***	—	2·14*	0·98	—

Significance: * 5 % level = 2·04; ** 1 % level = 2·75; *** 0·1 % level = 4·00.

a maximum on 7 July when there were an average of forty-three unhatched eggs and thirty-nine larvae per plant, and the treatments were applied on 14 July when the level of the attack had fallen to seven unhatched eggs and 22·5 larvae per plant. The sprays were applied by means of a knapsack sprayer, and the plants were treated individually, a drenching top and bottom cover being given. The weather had been hot and dry for almost a month up to the time of treatment, but rain started falling before the spray had time to dry.

Five days after treatment, on 19 July, a sample of thirty leaves was taken from each plot and the number of living and dead larvae per sample counted. The results obtained are shown in Table 7. There was a fairly heavy natural mortality of the larvae, but consideration of the numbers of living larvae per treatment shows the relative effectiveness of parathion and DDT-oil emulsion.

TABLE 7. *Comparison of parathion and DDT-oil emulsion against 2nd-generation larvae in the field; trial* 3, *Massingham, Norfolk*, 1949

Treatment	Wt./acre (oz.)	Living larvae 5 days after treatment: Treatment totals	Living larvae 5 days after treatment: % reduction control
Parathion, 0·002 % at 242 gal./acre	¾	105	71
DDT-oil emulsion, 0·125 % at 242 gal./acre	48	73	80
Control, water at 242 gal./acre	—	368	0

The high rate of application of the DDT-oil emulsion gave a good kill of larvae, considering that the majority of them were approaching maturity and that heavy rain fell immediately after treatment. The parathion at a similar high rate of application, but at a very low concentration, also gave a reasonably good kill under the same conditions. The high larval mortality at the time of treatment was presumably due to the extremely hot dry weather experienced before treatment which caused the plants to wilt badly.

Field trials nos. 4 *and* 5

In 1950 two field trials were carried out, the first at Burgh St Peter (Trial 4) and the second at Corton (Trial 5), both near Lowestoft, Suffolk, and some 5 miles apart. Details of the trial sites and the treatments applied are given in Tables 4 and 8 respectively.

As in trial 2 the sprays were applied by means of a small wheel-barrow-type spraying machine with 'high-volume' nozzles working at 60 lb./sq.in. set above the rows to give top cover only, and giving cones of spray 18 in. in diameter at ground-level. Compared with trial 2, where the cones of spray were only 6 in. in diameter at ground-level, the amount of spray falling on the plants in these two trials was a good deal less (about one-third as much) and more like that of commercial practice using machines designed for 'complete cover' rather than for 'row cover' only. The dusts were applied with a knapsack duster in a manner also approximating to commercial practice.

TABLE 8. *The treatments applied in trials 4 and 5*

	Insecticide	Concentration (%)	Rate/acre	Wt./acre (oz.)
Trial 4	Control, no treatment			
	DDT-oil emulsion	0·15	46 gal.	11
	DDT dust	5·00	110 lb.	88
	BHC suspension	0·02γ	44 gal.	2
	BHC dust	0·33γ	77 lb.	4
Trial 5	Control, no treatment			
	DDT-oil emulsion	0·15	50 gal.	12
	DDT-oil emulsion + synergist	0·05	54 gal.	4
	DDT dust	5·00	46 lb.	39
	Parathion dispersible liquid	0·01	52 gal.	¾

The plots were sampled before treatment and on the third, sixth and ninth days after treatment. Representative samples of thirty-six leaves were obtained in a random manner from each plot and placed in separate paper bags. The samples were examined in the laboratory within 24 hr. of collection.

Effect of treatment on the mangold-fly population. The effects of the treatments on the egg and larval populations at trials 4 and 5 are shown in Figs. 2 and 3. There were no significant differences in the infestation of the plots at either trial when the treatments were applied. Only at trial 5, was there any suggestion that treatment had affected the egg population. Here, on the third day after treatment, the plots treated with parathion carried slightly, but significantly, more unhatched eggs than did the other plots. The spray containing 0·15% DDT-oil emulsion gave significantly lower numbers of living larvae and higher numbers of dead larvae than the controls and other treatments in both trials on the third, sixth and ninth days after treatments, while 0·05% DDT-oil emulsion plus synergist gave a kill about one-third that of the ordinary DDT emulsion; roughly in proportion to the DDT content, which suggests that the synergist was without effect. In both trials, 5% DDT dust gave a poor kill, even when applied at the high rate of 110 lb./acre in trial 4. Parathion at the very low rate of ¾ oz./acre also gave only a partial kill of larvae. BHC applied both as dust and spray was ineffective.

At the site of trial 5, the remainder of the field outside the trial area was sprayed by the farmer with 3·0% DDT-oil emulsion at 5 gal./acre, 1½days after the trial treatments were applied. This 'low-volume' treatment gave a degree of control comparable with that given by 0·15% DDT emulsion applied at a 'high-volume' rate. Twice as much DDT was applied (24 oz. against 12 oz./acre), but the convenience of the method more than made up for the extra expense.

An attempt was made to assess the residual toxicity of this 'low-volume' spray

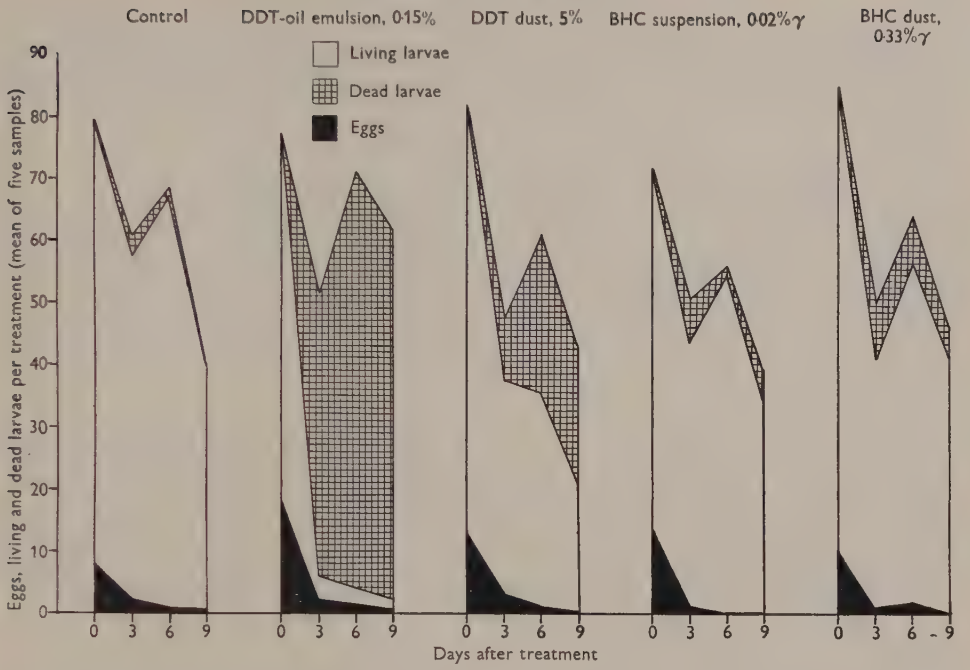

Fig. 2.

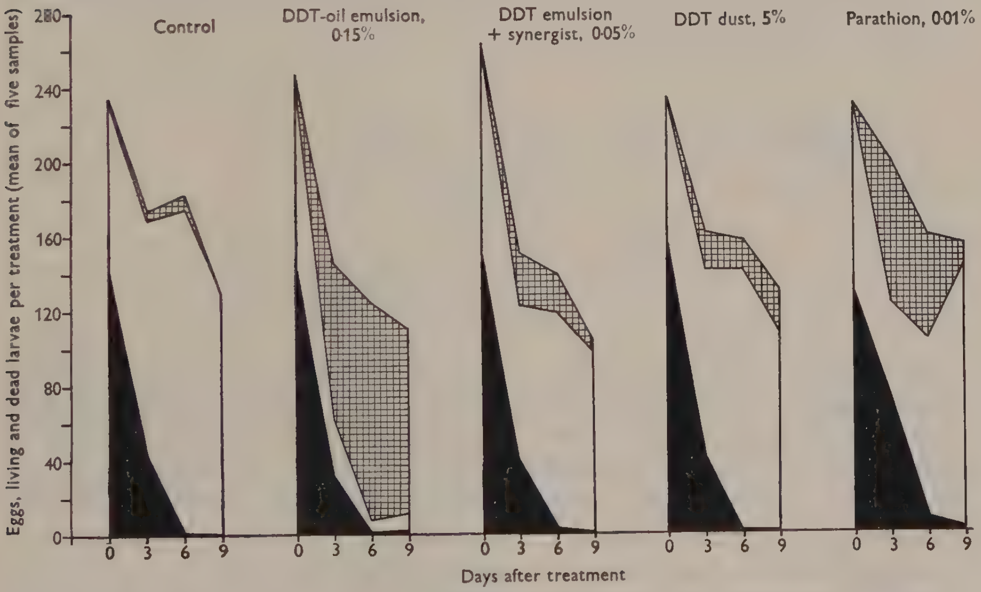

Fig. 3.

Figs 2, 3. The effects of insecticides on the egg and larval populations in trials 4 and 5.

on the leaves to flies of the second generation by caging newly emerged flies on leaves collected from the field 4 weeks after treatment. After 48 hr., there was no mortality in the controls and only 16% in the cages containing sprayed leaves in which the dead larvae, killed some 4 weeks previously, could still be seen. This small residual action is obviously of little practical value, especially as by this time most of the treated foliage had been replaced by new, untreated foliage. Even though a good kill of larvae was obtained on the 10-acre field as a whole, a second generation attack occurred at a moderate level, maximum counts of three eggs and eight larvae per plant being obtained on 11 and 19 July respectively. Clearly a very high kill of the first generation is necessary to eliminate subsequent generations, and even then there is always the possibility of flies coming in from neighbouring beet fields.

Effect of treatment on the plants. In addition to the counts of eggs and larvae made on the leaf samples from each plot in trials 4 and 5, marks were awarded to these samples for the effects of treatment on the leaves. Fifteen marks were awarded for samples of clean leaves and three marks for samples of badly mined leaves, with intermediate marks according to the degree of mining. Thus, the maximum possible marks for the five plots of each treatment was 75 and the minimum 15. The results of marking are given in Table 9.

TABLE 9. *Grading of treatments at trials 4 and 5 according to degree of mining: 9th-day samples. Total marks for five replicates. (Maximum marks 75 for the least damage)*

Trial 4		Trial 5	
Burgh St Peter	Maximum 75	Corton	Maximum 75
DDT-oil emulsion	71	DDT-oil emulsion	70
DDT dust	38	DDT-oil emulsion + synergist	25
BHC suspension	31	DDT dust	21
BHC dust	27	Parathion	22
Control	26	Control	16
		Farmer's 'low-volume' application of DDT-oil emulsion 1½ days later than above treatments	61

At the end of the attack by the first generation of mangold fly, about 2 weeks after treatment, two independent observers were asked to grade the plots. In each of the five blocks in both trials they were able to pick out the plots which were treated with 0·15% DDT-oil emulsion, because of the considerably improved growth of beet on these plots, but were unable to distinguish between the controls and the other treatments. The improvement from treatment with DDT-oil emulsion was most marked in trial 5 where the attack was heavier.

Effect of treatment on yield. The visual effects at trial 5 suggested that the successful treatment with 0·15% DDT-oil emulsion would be likely to result in an increase in yield. Accordingly, the plots were harvested and yield data obtained.

Although the plots treated with 0·15% DDT-oil emulsion gave the highest yield of roots and sugar, statistical analysis showed that there were no significant differences resulting from treatment.

Field trials nos. 6 *and* 7

In 1952, two more field trials were undertaken in which only 'low-volume' sprays were used. In trial 6, a range of insecticides of the chlorinated hydrocarbon class, all formulated in oil as emulsions or miscible liquids, was applied at 10 gal./acre and at a concentration of the active ingredient to give 6 or 24 oz./acre). In trial 7, one standard DDT-oil emulsion was employed at rates of 5 and 10 gal./acre, the concentration being varied in each case to give 6, 12 and 24 oz. DDT/acre. The sprays were put on with a small engine-driven 'low-volume' sprayer mounted on a Land Rover, the speed of which was controlled by a governor. Details of the trial sites are given in Table 4.

Effect of treatment on mangold-fly larvae. At both trial sites, the egg and larval populations were estimated immediately before treatment and again after 6 days, samples of forty and sixty leaves per plot being taken at trials 6 and 7 respectively. Statistical analysis of the figures from both trials showed that the egg and larval populations were randomly distributed before treatment and that treatment had not caused any change in the distribution of the total population (eggs, living and dead larvae) after treatment. Evidently there had been no abnormal loss of larvae by migration from the leaves on sprayed plots, and the counts of dead larvae were satisfactory. The results, in terms of dead larvae percentage of total larval population, are presented in Tables 10 and 11.

TABLE 10. *Effect of treatments in trial* 6, *Norton*, 1952: *percentage of dead larvae* 6 *days after treatment*

Treatment				Effect
Insecticide	Type	Rate of application	Oz./acre	% of dead larvae
Control	—	—		5·5
DDT	Stock emulsion	All at 10 gal./acre	24	97·0
DDT + synergist			24	96·3
BHC	Miscible oil		6	87·5
Lindane			6	91·8
Toxaphene			24	91·5
Aldrin			6	84·5
Dieldrin			6	94·0
S.E. (spray treatments only)				±3·8

All treatments were highly successful and gave figures so different from the controls that their significance was clear without statistical analysis. In both trials, however, analysis of the effects of the treatments alone failed to reveal significant differences between them. Inspection of sample totals indicated that variability

TABLE 11. *Effects of treatment with DDT in trial 7, Gorleston 1952: percentage of dead larvae 6 days after treatment*

Treatment				Effect	
Insecticide	Gal./acre	Concentration (%)	Wt./acre (oz.)	% dead larvae	
Control	—	—	—	0·8	
DDT-oil emulsion	10	1·5	24	94·0	91·4
		0·75	12	91·8	
		0·37	6	88·5	
	5	3·0	24	90·8	87·0
		1·5	12	85·3	
		0·75	6	85·0	
	s.e. (spray treatments only)			±2·9	±1·7

was introduced largely as a result of occasional leaves being missed by the sprayer. This arose not so much from the failure of the sprayer as from the sheltering effect of one leaf upon another. When allowance for this variability had been made, it was possible to arrange results in sequence as follows:

	Insecticide		Oz. insecticide/acre	Kill (%)
Trial 6	Dieldrin	(All at 10 gal.)	6	98
	DDT		24	96–97
	BHC		6	92–93
	Toxaphene		24	92
	Aldrin		6	89
Trial 7	DDT			
	10 gal./acre		24	94
			12	92
			6	89
	5 gal./acre		24	91
			12	88
			6	85

The contact effect of spray deposits upon migrating larvae. Using sprayed leaves collected from trial 6, attempts were made to assess the contact effect of the spray deposits upon migrating larvae. The sprayed leaves from each treatment were placed separately in small dishes and four batches of five larvae were placed upon each set of leaves. The larvae were left for 15 min. during which they wandered about and usually began to attempt re-entry. Clearly any effect of the insecticides on the larvae would be mainly but not entirely by contact action. After exposure, the larvae were transferred to honey jars and supplied with clean leaves. Mortality was estimated after 2¾, 5½ and 16 days. Between the sixth and sixteenth days many of the surviving larvae pupated and, when pupation was complete, the pupae were opened to ascertain their condition. The results are shown in Fig. 4. Dieldrin caused the greatest mortality, aldrin the next greatest followed closely by DDT + synergist. The remaining treatments, including DDT without synergist, were less effective, all causing a final mortality of about 30%.

It will be noted that the order of effectiveness is somewhat different from that shown when the sprays act upon larvae in mines. There is, for example, a noticeable difference in the efficacy of the DDT preparations, that with the synergist giving an enhanced kill.

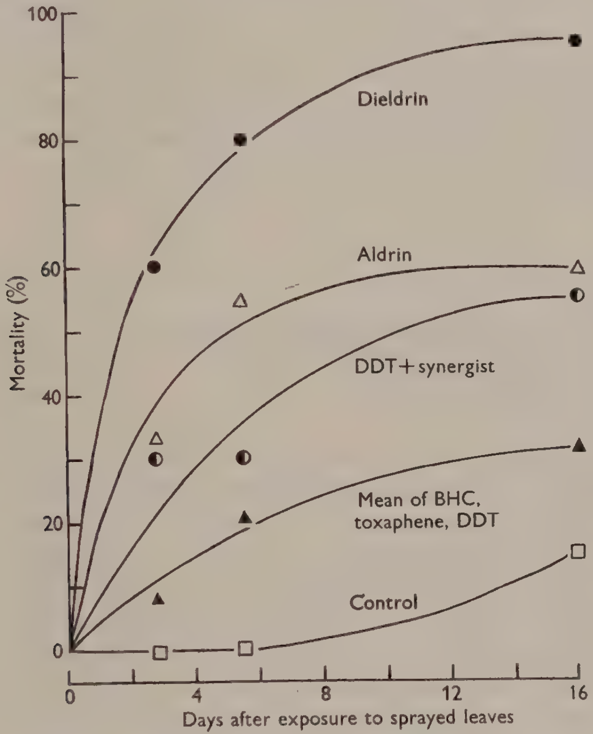

Fig. 4. The contact effect upon larvae of insecticides applied in trial 6.

SPRAY PHYTOTOXICTY

Experience in the application of insecticidal sprays to sugar beet has shown that commercial products of the 'stock emulsion' and 'miscible oil' types may cause injury to the plants. This is generally negligible when 'high-volume' spraying is employed, except where the rate of application is excessive or the concentration of the insecticide, and therefore of its oil carrier, is abnormally high. Thus in field trial 1 at Ramsey in 1949, considerable scorch was noticed with 1 and 0·5% DDT-oil emulsion applied at 110 gal./acre. The risk of injury is greatly increased when 'low-volume' applications are made, since a high concentration of the insecticide is then essential. Accordingly, in 1951, a field trial was undertaken to test a number of commercial products containing DDT and other insecticides of the chlorinated hydrocarbon class. Ten products were employed, each at three different concentrations and three different rates of application. Thus, there were nine different treatments for each product and ninety plots in all. Each plot consisted of 11 yd. of row in a commercial beet crop, taking in about thirty to forty plants, and separated from neighbouring plots by a discard row. Treatments were applied when the plants were in the 4 to 6-foliage leaf stage, shortly after the crop had been singled. The row width was 23 in. and the 'low-volume' nozzle was set so that the 'cover' extended to the centre of the inter-row on either side of the treated plants. Treatments were applied in hot sunny weather which continued until rain fell on the fifth day after application. The plots were graded on the fifth, tenth and twentieth day after treatment according to the following system, notes also being made on the presence or absence of leaf curl and distortion:

Marks	
0	Total destruction of plants
1	Very bad scorching, some leaves destroyed and plants severely injured, but recovery probable
2	Pronounced scorching, with appreciable injury to plants
3	Scorched spots and blotches, injury slight
4	Scorched spots just detectable, injury negligible
5	No apparent injury

Symptoms of scorching were obvious within a day or so of treatment on some plots but had largely disappeared by the twentieth day. Table 12 gives details of the products applied and the grading of the plots on the tenth day, when the injury was at its worst. Only three products (A, B and J) were acceptable over the whole range of concentrations and dosages, three others (C, E and H) were acceptable at the lower ranges, while the remaining products (D, F, G and I) caused injury over almost the whole range. These latter products must be regarded as unsafe for use on sugar beet unless they give control of pests at concentrations below 1 % of the active ingredient. Products A and B, at 4 % of the active ingredient and 10–20 gal./acre, are near the limits of usefulness, but these rates are high for commercial practice.

This trial emphasizes the need for care in the choice of product for 'low-volume' spraying of sugar beet. The oil in the emulsion is most probably responsible for scorch; products A, E and H all contain solvent naphtha, though not necessarily of the same grade, and all three are reasonably satisfactory in performance. A and B were the products utilized in the laboratory and field experiments already considered. The remaining products contain a variety of other organic solvents.

DISCUSSION OF RESULTS

The results of the early laboratory experiments and field trials (nos. 1, 2 and 4) in which various formulations of BHC and DDT were tested for the control of larvae in mines suggested that DDT was superior to BHC at customary rates of applica-

TABLE 12. *Effects of commercial sprays of the stock emulsion and miscible oil type at low-volume rates on beet: tenth day after treatment. Beet in 8-leaf stage and damage confined to outer 4 leaves*

			Concentration and rate of application of insecticide									
			4 %			2 %			1 %			
Key letter	Product	Insecticide	20 gal. per acre	10 gal. per acre	5 gal. per acre	20 gal. per acre	10 gal. per acre	5 gal. per acre	20 gal. per acre	10 gal. per acre	5 gal. per acre	Score (max. 45 for least damage)
A	Psylortox, 167	DDT	4·8	5	5	5	5	5	5	5	5	44·8
B	Arkotine, D. 15	DDT	3 M.D.	4	5	5	5	5	5	5	5	42·0
C	Arkotine, S.D. 18	DDT	1 M.D.	2	3	3	4·5	5	5	5	5	33·5
D*	Arkotine, S.B. 25	BHC	0·5	1·5	3	1 B.D.	2 M.D.	4	3 M.D.	4	5	24·0
E	DDT miscible liquid	DDT	2·5	3	4·5	4·5	5	5	5	5	5	34·5
F	Toxaphene, 25 %	Toxaphene	0·5 B.C.	2	3	1·5 B.C.	2	3·5	2·5	3·5	4·5	23·0
G*	Kerex Arkotine	BHC	1 B.C.	1·5 B.C.	3 S.D.	2·5 M.D.	4	4·5	3·5 S.D.	4·5	5	29·5
H*	Gammacide, C.L.	BHC	1·5 B.D.	2·5 M.D.	4	4 S.D.	5	5	4	5	5	36·0
I	Aldrex 2	Aldrin	1 B.D.	1·5 M.D.	2·5 M.D.	2·5 M.D.	4	4·5	3	5	5	29·0
J	Dieldrix 15	Dieldrin	5	5	5	5	5	5	5	5	5	45·0

S.D., M.D., B.D. = slight, moderate or bad general distortion of plant.
S.C., M.C., B.C. = slight, moderate or bad leaf curl.
H* was used at 1, 0·5 and 0·25 % of active constituent, γ-BHC.
D* and G* were used at 4, 2 and 1 % crude BHC corresponding to 1, 0·5 and 0·25 % γ-BHC.

tion and that, of the DDT formulations, DDT-oil emulsion was better than wettable powders or dusts, which gave good results only when applied at excessive rates. Unfortunately, at this stage, no BHC-oil emulsion was employed.

Parathion was extremely effective when used in the laboratory as a spray at 0·01% giving a complete cover. In the field at the same concentration and at 52 gal./acre, it gave a poor kill when applied as a top cover only (trial 5), whereas, when applied as a complete cover at 0·002% and a rate of 242 gal./acre (i.e. at an equivalent amount of $\frac{3}{4}$ oz. parathion/acre, trial 3) it gave a fair kill of second-generation larvae mining in large leaves. D. Price Jones (*in litt.*) has obtained excellent kills of larvae in the field with 'high-volume' and 'low-volume' sprays using as little as 2–4 oz. of parathion/acre. Moreover, when successful, the kill from parathion is more rapid than from DDT. None of the insecticides used, with the possible exception of parathion, appeared to have any effect on eggs.

In the later field trials (nos. 5–7), effective commercial control was obtained with a DDT-solvent naphtha emulsion at 'high'- and 'low-volume' rates, the spray being applied as a top cover only at from 6 to 24 oz. DDT/acre. Satisfactory kills were obtained ranging from 85 to 97% on the sixth day after treatment. Despite the slower kill and greater weight of DDT per acre necessary, DDT compares favourably with parathion in price and is much safer to handle. In Trial 6, effective control was also obtained with other chlorinated hydrocarbons formulated as oil emulsions, viz. BHC, toxaphene, aldrin and dieldrin. All were applied at 10 gal./acre giving top cover only, the quantity of insecticide used being 6 or 24 oz. acre.

Ernould (1948), in Belgium, found that DDT and BHC dusts killed the flies, and that wettable BHC and BHC-oil emulsion killed 73–100% of the larvae in the leaves within 3 days of treatment, while DDT-oil emulsions had a slower action and killed 95–100% of the larvae within 6 days of treatment. The best wettable DDT preparations killed only 50–70% of the larvae within 3 days. White (1947) used DDT and BHC dusts against three different stages; adult flies emerging from old beet fields, eggs laid on seedling beet, and larvae mining in leaves. Control of eggs and flies was unsatisfactory, but good kills of larvae in mines were obtained on a small scale.

J. R. W. Jenkins and J. Morgan Jones (*in litt.*) carried out trials in 1948 at four different centres in Wales, using BHC dust against larvae mining very small plants (cotyledon stage to two foliage leaves) in late May and early June. Counts of larvae, made at intervals after treatment, showed no significant differences, although visual assessment suggested that the degree of control obtained was more satisfactory than the counts indicated.

In 1948, Miss E. Britton (*in litt.*) used $2\frac{1}{2}$% BHC dust at 1 cwt./acre against freshly hatched larvae mining very small plants (cotyledon stage). A good control was obtained, the figures from counts being significantly different. No control of the second-generation larvae was given by $3\frac{1}{2}$% dust at 60–70 lb./acre. Anon. (1951)

advised the destruction of larvae in leaves by means of sprays based on DDT-oil emulsion, wettable BHC, or parathion.

The successful control obtained by the use of BHC dust or wettable powder in some of the above instances may be due to the small size of the plants treated. Thus, those treated by Britton on 21 May, 1948 were very backward, being in the cotyledon stage at the time. The same is true of the plants treated by Jenkins and Morgan Jones, which possessed only cotyledons, or cotyledons plus two foliage leaves, in late May and early June. Ernould also drew attention to the smallness of the plants in Belgium at the time of heavy attacks in 1947, when his observations were made. In the field trials conducted by the authors the plants possessed two cotyledons and about six foliage leaves at the time of treatment and were normal for the time of the year. In the laboratory trials, leaves treated were of equivalent size. When plants are small there may be a greater tendency for larvae to wander and therefore to make contact with external films of insecticide, although this may not be the complete explanation of the successful control sometimes claimed for formulations of BHC not containing oil.

Kerr (1951), working on three species of leaf miner, found that aldrin and dieldrin were the most effective insecticides, whilst lindane, chlordane and DDT were less effective, the degree of control varying considerably from species to species. He considers that these insecticides should be regarded as chemotherapeutic agents. Morris (1950) tested formulations of DDT, BHC and DNOC against *Syringopais temperatella* Led., a Tineid leaf-miner of cereals in Cyprus. The control obtained with DDT and BHC was thought to be due to migration of larvae, whereas DNOC was considered to act directly on the larvae concealed in mines. In our experiments there was virtually no migration of small larvae and yet they were killed within the leaf by DDT and parathion. The mode of entry of the insecticide remains in doubt. The vapour pressure of DDT is so low that gaseous diffusion through the stomata seems unlikely to be sufficient. More rapid gaseous diffusion would be expected from BHC with its higher vapour pressure, but BHC was no more effective than DDT when used at the same rate (trials 6 and 7). Some ingress of sprays might occur through stomata, but this explanation also has little to support it as DDT dust, heavily applied, gave a considerable mortality. Moreover, 'low-volume' DDT spray at 5 gal./acre gave an effective kill, even though the cover was very discontinuous. For these reasons, it seems probable that actual penetration of the leaf tissue may take place. The greater efficiency of DDT-oil emulsion over wettable DDT and of DDT dust plus oil over ordinary DDT dust and the success of BHC-oil formulations in trial 6 suggests that oil formulation gives better contact with the leaf surface and improves penetration. The addition of a resinous synergist to DDT-oil emulsion did not appear to enhance its action, although direct comparison with the DDT-solvent naphtha emulsion was impossible because the DDT + synergist was made up in another oil. The resinous synergist may affect the physical state of the DDT in the surface film without materially affecting its rate of penetration of leaf tissue. This conclusion is supported by the experiment carried out in conjunction with field trial no. 6, in which an attempt was made to assess the contact action of various chlorinated hydrocarbons on free larvae. Here the order of toxicity was different from that to larvae in mines and the synergist in one of the two DDT preparations appeared to have enhanced the kill.

A kill of 90–95% of larvae in mines is commercially satisfactory and is likely to permit the survival of small populations of parasites which may have the opportunity of increasing with subsequent generations of the fly. A kill of this order can be achieved with relatively small amounts of DDT (trial 7, Table 11) or parathion (D. Price Jones, *in litt.*) applied as a discontinuous cover to the upper surface of the leaves only. In all probability reduced amounts of dieldrin (i.e. less than 6 oz./acre) would also be effective, but it is doubtful whether toxaphene could be reduced much below 24 oz./acre or γ-BHC and aldrin below 6 oz./acre. Biologically, the best control measure is the one which employs the smallest amount of the insecticide least damaging to parasites and other beneficial insects. In this respect the addition of a resinous synergist to DDT is undesirable because it appears to improve contact action of the residual film without influencing the kill of larvae in mines. The vigorous contact action of dieldrin and aldrin against mangold-fly larvae (Fig. 4) also suggests that these two insecticides might be more harmful than DDT, BHC or toxaphene to parasites, although this supposition lacks proof. In a normal season, larval parasites attacking larvae in the leaves account for 70–75% of the total parasitism, the principal species being *Biosteres carbonarius* Nees (Braconidae). It is a fortunate circumstance that the larval parasites operate mainly from the lower surfaces of the leaves. Thus, the application of insecticide as a discontinuous cover to the upper surfaces of leaves probably permits a greater survival of parasites than might otherwise be the case.

In the 1950 field trials attempts were made to assess the degree of defoliation caused by the mangold-fly larvae and to relate it to the larval populations and to the effect on yield. The maximum defoliation does not necessarily coincide with the peak number of mining larvae per plant. It is determined by two opposing processes, the rate of destruction of old leaves and the rate of production of new leaves. Both processes are influenced considerably by climatic and edaphic factors which vary from field to field and season to season. In trial 5, maximum defoliation reached about 50%, the population being ten eggs and fourteen larvae per plant when the plants possessed two cotyledons and six foliage leaves. Corresponding figures for trial 4 were 30% defoliation and a population of six eggs and nine larvae per plant when the plants possessed two cotyledons and six foliage leaves. In trial 2, where there was a population of 24·8 unhatched eggs and 22·9 larvae per plant when the plants possessed two cotyledons and six foliage leaves, the percentage defoliation was not determined, but must have reached 70% or more. In all three trials treatment with DDT-oil emulsion controlled the larvae and gave very obvious differences in the size of treated plants compared with controls. The determination of treatment yields showed that defoliation at the 70% level produced a decrease in yield of about ¾ ton/acre (cash value £3. 15*s*. 0*d*.), whilst at 50 and 30% defoliation there appeared to be no significant reduction in yield.

Experiments are being conducted in which the plants are artificially defoliated to varying degrees. In these experiments the degree of defoliation can be decided in advance, and no disturbing factors are introduced such as the incidental control of other subsidiary pests which may happen in field trials with insecticides against defoliating insects. Results of the first three trials conducted in 1951 indicate that only defoliation in excess of 50% seriously affects the yield of roots and sugar in an ordinary sugar-beet crop. It may be concluded that control measures are only worth while if the crop is backward or if the egg and larval populations per plant are exceptionally high.

RECOMMENDATIONS FOR CONTROL

Preventive measures, such as the siting of beet fields as far away as possible from the old ones and the preparation of a good seed-bed with suitable manuring, should be followed. Early sowing is also desirable to ensure that plants have several rough leaves at the time of attack. Drilling after the middle of May avoids attacks by the first generation, but such a drastic measure would rarely be necessary and could not be advocated as a general policy since it would lead to serious losses of yield in years when mangold fly was not prevalent. If an attack develops, gap only and delay singling, or delay both gapping and singling until oviposition has ended.

In a normal year plants should be examined in the second and third weeks in May. If there are more than about thirty unhatched or hatched eggs per plant in the 6 to 8-leaf stage, or proportionately fewer if the plants are smaller or not growing well, direct control measures are worth while. The plants should be sprayed with one of the following preparations:

(1) 0·15% DDT-oil emulsion at 50 gal./acre (12 oz./acre).
(2) 0·75–1·5% DDT-oil emulsion at 10 gal./acre (12–24 oz./acre).
(3) 2–3% DDT-oil emulsion at 5 gal./acre (16–24 oz./acre).
(4) 1·5% toxaphene-oil emulsion at 10 gal./acre (24 oz./acre).
(5) 0·375% aldrid, dieldrin or γ-BHC in oil emulsion at 10 gal./acre (6 oz./acre).

It is not necessary to concentrate the spray on the rows as only a top cover is required. Care should be taken to avoid using preparations that might cause scorch, and an assurance from the manufacturer should first be obtained on this point.

Parathion should be used at 2–4 oz./acre, but only by contractors with experienced spray crews. The use of 5% DDT dust (Dunning & Jones, 1950) can no longer be recommended.

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EXPERIMENTS ON SPRAYING WITH DDT TO PREVENT THE FEEDING OF *SCOLYTUS* BEETLES ON ELM AND CONSEQUENT INFECTION WITH *CERATOSTOMELLA ULMI*

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Experiments were done for three years on street elms at Folkestone and Aldenham to repeat American work on the use of insecticidal sprays to prevent feeding by *Scolytus* beetles, which carry *Ceratostomella ulmi*, the cause of elm disease. Although the fungus can remain alive in the older annual rings of an infected tree, active disease usually results from fungus freshly introduced by *Scolytus*. Thus spraying is of value on infected, as well as uninfected elms.

The results of the experiments supported American conclusions that DDT was superior to benzene hexachloride. A 1·5% solution of DDT in miscible oil gave reasonably good, but not perfect, control, and was much superior to solutions made from DDT in the form of a dispersible powder.

Spraying of trees 20–30 ft. high cost over £1 per tree per year, which is considered prohibitive, except for small trees of special amenity or sentimental value.

Introduction

In Europe the elm bark beetles, *Scolytus scolytus* F. (*S. destructor* Olivier) and *S. multistriatus* Marsham are chiefly responsible for spreading the fungus *Ceratostomella ulmi* Buism., the cause of the wilt disease of elms, known as the elm disease or sometimes as the Dutch elm disease.

There are several general descriptions of this serious disease (Forestry Commission, 1951; McCallum & Stewart, 1951; Walter, May & Collins, 1943), and here it is necessary to deal only briefly with the relationship of the insect to the fungus and to the disease. *Scolytus* beetles breed only in weakened or dying trees. Breeding galleries often occur in felled or windblown elm wood for a year or two after detachment, and in trees seriously weakened either by the fungus or by other causes such as flooding or drought. The female beetles make egg-laying tunnels in the inner bark contiguous to the wood, and when the larvae hatch they make secondary galleries, spreading from the egg tunnel. They finally emerge from these, after pupation, at any time from May to October. The microscopic fructifications, both asci and coremia, of *Ceratostomella ulmi* frequently occur in these beetle galleries, and a proportion of the beetles emerging from an infected elm carry spores of the fungus. The proportion of infected beetles can be as high as 70% (Parker, Collins, Tyler, Connola, Ozard & Diedrich, 1947). Before they breed, the young beetles feed on the twigs of healthy elms. Except in a very heavy attack, feeding is restricted to the crotches of the twigs, where a small tunnel or groove is made. It is at this point that infection usually takes place, any time between mid-May and the end of August (Banfield, 1948). Early infections are likely to be more serious, as the fungus has then a longer period to spread in the tree. At whatever time of year infection takes place, the fungus does not normally occur in the later-formed summer wood of the annual rings. For this reason, in young trees at any rate, it fails to pass from one annual ring to the next (Banfield, 1947), unless infection is so severe that it reaches the roots, where the annual rings are less clearly defined and the passage of the fungus is easier. Banfield, Rex & May (1947) have suggested that the fungus is more likely to reinfect the next annual ring in larger trees than in small ones, but field data collected in Great Britain (as yet unpublished) show that the disease often fails to re-occur even in large infected trees.

As soon as the role played by *Scolytus* was realized, measures were taken against the insect, as well as the fungus. The felling of diseased trees, or the removal of dead and dying wood from them, was mainly aimed at reducing the infected-beetle population. Various chemical treatments were suggested for felled timber to prevent beetles breeding in it. It was not until 1946, however, that work was started on the prevention of beetle feeding as a means of protecting elms from infection. Early experiments (Becker, 1947), in which DDT was one of the substances tried, were not very successful, but subsequent work was more promising (Diedrich & Balin, 1949; Dimond, Plumb, Stoddard & Horsfall, 1949; Plumb, 1950; Whitten, 1948). In America recommendations for spraying with oil emulsions of DDT are now generally included as one means of protecting elms from *Ceratostomella* (McCallum & Stewart, 1951; Swingle, Whitten & Brewer, 1950).

In 1947 Curtis May, of the United States Division of Forest Pathology, sent the author a note on trial formulations of DDT dissolved in benzene, xylene and various proprietary oils, which were being tried by the Bureau of Entomology and Plant Quarantine to protect elms from *S. multistriatus*, the most important vector of the fungus in the United States. Further details on materials and strengths were provided by D. E. Parker and R. R. Whitten of that Bureau. Although it was realized, even then, that spraying of this kind could play only a limited part in the control of the disease, it was decided that similar experiments ought to be done in Great Britain. The Americans had reported better results with DDT in oil emulsion than as a water dispersible powder, or than with benzene hexachloride in any form. However, in consultation with the Jealott's Hill Research Station of Imperial Chemical Industries, who kindly supplied the necessary materials, it was decided to cover a reasonably wide range of formulations in the first year's experiments, which were made in 1948.

Experimental work, 1948

At that time the disease was doing considerable damage to street elms in Folkestone, the amount of infection being increased by invading beetles from neglected elms on neighbouring private property. The affected trees, which were 20 to 30 ft. high, were very suitable for an experiment of this kind, and the Parks Department kindly offered to assist with the spraying.

The American workers suggested two sprayings, the first to be given while the leaves were expanding, but not yet big enough to shield the twigs from spray, and the second $2\frac{1}{2}$–3 months later. At Folkestone the trees were barely in leaf when sprayed the first time on 19–22 April, and the second spraying on 26–29 June, followed after a rather short interval. The trees sprayed were spaced irregularly in avenues along residential streets. Some diseased elms had already been felled, and some remaining were badly affected. A few had *Scolytus* beetles breeding in the trunk, and were therefore useful sources of infected beetles, as were similar trees in neighbouring streets. The elms sprayed were situated in two areas just over a quarter of a mile apart, in one case along a single road, and in the other along three contiguous roads. In tabulating results the two areas are considered together. Forty-eight trees were sprayed, normally six with each treatment, and thirty-three were left unsprayed as controls, three of which were considered to be too badly diseased to be worth spraying. They are not included with the rest of the controls in the tables below. The controls were not selected strictly at random. Some trees were left because they were badly situated for spraying, being intermingled with other trees, or above choice plants in neighbouring front gardens. This affected about one-third of the controls. The remainder were selected to give a reasonable distribution throughout the length of the avenues treated.

The spraying was done with a small pressure sprayer and a long lance. It appeared that a reasonable coverage was obtained, except in the extreme top of the tree, which is, unfortunately, a particularly vulnerable point for infection. Some spray certainly reached the top, though it was not possible to be sure that coverage was complete. Some trouble was experienced with wind drift. The trees were examined on 20 August, and again on 24 September, and classified by eye according to the current year's symptoms of *Ceratostomella* attack. The tables below are compiled from these two assessments combined.

TABLE 1. *Recurrence of active symptoms at Folkestone in* 1948

	Treatment in 1948	No. of trees	No. showing active symptoms in 1948
Trees showing symptoms in 1947	Controls	9	9
	Sprayed	16	5
Trees not showing symptoms in 1947	Controls	21	15
	Sprayed	22	9

It was still possible, when the trees were numbered during the winter of 1947–8, to judge very roughly the severity of attack in 1947 by the characteristic 'shepherd's crooks' at the tip of dead twigs, dead wood, etc. These observations form part of the basis of Table 1, showing the extent to which active symptoms recurred.

These figures on the whole support the contention that fresh infection with the fungus *Ceratostomella* is usually required if an elm is again to develop active external symptoms; for spraying greatly reduced the proportion of trees showing symptoms for the second year in succession. Obviously spraying could act only by preventing fresh infection; it could have no effect on the movement of the fungus from one annual ring to the next within the tree.

The materials used, DDT and BHC, were both applied as a dispersible powder and in a miscible oil. They were diluted with water to give the actual percentage of DDT or γ-BHC isomer shown in Table 2. The amount of diluted spray required per tree varied from 3 to 6 gal. and averaged about 4·5 gal. The stronger solution of DDT used approximated to the recommendations received from America, and the weaker solution was included as being less liable to damage foliage, and cheaper. The stronger solution of BHC was the highest concentration considered reasonably safe.

In Table 2 the effect of the spraying on infection and re-infection is set out. Eight severity ratings were used in the assessment of the symptoms:

0 None.
1 Very slight. Only a few twigs affected.
2 Slight. Only twigs affected, but more general.
3 Moderate. Very general withering of twigs, or one branch moderately affected.
4 Rather severe. At least one branch severely affected.
5 Severe. Several branches severely affected.
6 Very severe. All or nearly all the tree severely affected.
7 Dead.

The tables compare the different treatments by adding up the rating score (i.e. one tree at 3 and one at 6 makes 9, or two trees at 2 and two at 4 makes 12), and dividing by the number of trees.

Table 2. *Effect of spraying elms at Folkestone in* 1948

Spray		No. of trees	Ratings								Total ratings	Average rating
			0	1	2	3	4	5	6	7		
DDT powder	1·5 %	6	5	—	—	1	—	—	—	—	3	0·5
DDT powder	0·75 %	6	3	1	1	—	1	—	—	—	7	1·2
DDT oil	1·5 %	6*	4	—	—	1	—	—	1	—	9	1·5
DDT oil	0·75 %	6†	5	—	—	—	—	—	1	—	6	1·0
BHC powder	0·1 %	5	2	—	—	1	1	—	1	—	13	2·6
BHC powder	0·05 %	7	3	—	2	—	2	—	—	—	12	1·7
BHC oil	0·1 %	6	4	—	—	1	—	—	1	—	9	1·5
BHC oil	0·05 %	6	4	1	—	1	—	—	—	—	4	0·7
Controls		30	6	2	5	7	1	2	6	1	90	3·0

* Owing to shortage of materials no trees received the second spraying.

† Owing to shortage of materials only three trees (all rating 0) received the second spraying.

There were too few trees for satisfactory statistical analysis, even though the distribution of the treatments was randomized. But there is evidence in favour of spraying, and weaker evidence favouring DDT in comparison with BHC. This agrees with American experience, where the initial toxicity of BHC was satisfactory, but it did not give such lasting protection as DDT. DDT in miscible oil may have given better results if there had been enough material available to complete the second spraying. Failure to complete the sprayings invalidates any comparison between miscible oil and dispersible powder as far as DDT is concerned; but the figures tend to show that BHC in miscible oil is superior to BHC dispersible powder.

EXPERIMENTAL WORK IN 1949

It was decided to repeat the experiment in 1949 using more trees, but only DDT as dispersible powder and miscible oil was used. As the 1·5% solution had not damaged foliage in 1948, a slightly stronger solution (2%) was included. The main experiment was again carried out on street trees at Folkestone, but a secondary one, using only one strength of DDT, was done on rather smaller elms in an avenue planted in the lawn in front of Aldenham House, near Watford, by kind permission of the Aldenham Estate and the British Broadcasting Corporation. Some tall, old elms in the avenue were not included in the experiment; but several of them showed symptoms of elm disease and abundant dead wood, and they probably acted as a source of infected beetles. In both places the work was done by contract, using a power sprayer.

Owing to various difficulties the first spraying was later than in 1948. At Aldenham it was done on 1 May, when the trees were rapidly coming into leaf, and some active beetles had been observed. At Folkestone it was not done till 6 and 7 May, but there, possibly because of the cooler maritime climate, the trees were more backward and no active beetles were observed. The power sprayer used a larger volume of spray, but probably gave a better coverage. At Folkestone considerable difficulty was experienced with spray drift on to houses and gardens. The second sprayings were given on 19 July at Folkestone and on 20 July at Aldenham, which was rather nearer to the interval between first and second sprayings suggested by the American workers. Assessments were made at Aldenham on 9 September, and at Folkestone on 13 September.

At Folkestone forty-seven of the trees sprayed in 1949 had also been sprayed in 1948, some with a different substance or a different strength. The other sixty were in streets not included the previous year, and, as before, the controls were selected partially to avoid spraying awkwardly-placed trees, and partially to get reasonable distribution. The selection of these additional trees was left too late for observations on their disease condition in the previous year. The condition of the original forty-seven trees and the controls mixed with them was known from the assessments made in August and September 1948, and forms the basis of Table 3.

TABLE 3. *Recurrence of active symptoms at Folkestone in* 1949

	Treatment in 1949	No. of trees	No. showing active symptoms in 1949
Trees showing symptoms in 1949	Controls	21	4
	Sprayed	17	6
Trees not showing symptoms in 1948	Controls	7	4
	Sprayed	30	9

The proportion of active infections in 1949 was lower than in 1948, in fact so low as partially to invalidate the whole experiment. Also only a very small proportion of the controls which had active disease in 1948 were again active in 1949, additional evidence that transfer of the fungus from one annual ring to the next within the tree is the exception rather than the rule.

The assessment was worked on the same rating as the previous year, except that, in view of the very small number involved, severe and very severe were grouped together and given a rating of four points. The results are set out in Table 4.

TABLE 4. *Effect of spraying elms at Folkestone in* 1949

Spray	No. of trees	Rating					Total ratings	Average rating
		0	1	2	3	4		
DDT powder 2·0 %	18	15	1	—	2	—	7	0·39
DDT powder 1·5 %	18	17	—	—	1	—	3	0·17
DDT powder 0·75 %	17	11	—	3	3	—	15	0·88
DDT oil 2·0 %	18	13	1	2	2	—	11	0·61
DDT oil 1·5 %	18	12	—	3	2	1	16	0·89
DDT oil 0·75 %	18	15	—	1	2	—	8	0·44
Total sprayed	107	83	2	9	12	1	60	0·56
Control	71	57	3	4	5	2	34	0·48

Again the figures cannot be statistically analysed, but obviously spraying had little or no effect. The low percentage infection would have greatly lowered the value of the results, but the failure of the spraying to control the few infections that did occur is unexplained.

At Aldenham a preliminary examination of the trees was made on 20 October 1948, when it was still possible to judge, with a moderate degree of accuracy, which trees had been actively attacked in that year. Table 5 is based on these observations and on the observed development of the disease in 1949.

TABLE 5. *Recurrence of active symptoms at Aldenham in* 1949

	Treatment in 1949	No. of trees	No. showing active symptoms in 1949
Trees showing symptoms in 1948	Controls	16	6
	Sprayed	17	1
Trees not showing symptoms in 1948	Controls	13	5
	Sprayed	23	1

Again these figures do not suggest that there are many trees where active attack in one year leads directly to active attack in the next. Included in these totals are twelve trees, which, because of severe attack in 1948 and earlier years, had very little live crown left. As an experiment, these were cut back very severely in March 1949, as much dead wood as possible being removed. Five of these were sprayed and seven left as controls; only one, a control, redeveloped the disease in 1949.

There were six rows of trees in the entire planting, and it was originally intended to include in this experiment only the two outer rows on each side, which consisted solely of small elms. The two centre rows were originally of an earlier planting, and the surviving old elms were the large trees already mentioned which it was hoped would provide infected beetles. Some of these had been removed and twenty-four of the small elms, which had replaced them, were used as additional controls. As no observations had been made on their health in 1948, they do not appear in Table 5. The assessment ratings used were the same as those at Folkestone.

TABLE 6. *Effect of spraying elms at Aldenham in* 1949

Spray	No. of trees	Rating 0	1	2	3	4	Total ratings	Average rating
DDT powder 1·5 %	20	18	—	—	1	1	7	0·35
DDT oil 1·5 %	20	20	—	—	—	—	0	0
Total sprayed	40	38	—	—	1	1	7	0·18
Controls	53	34	5	6	6	2	43	0·81

In the experiment recorded in Table 6 the spraying provided a very high degree of control, admittedly under conditions where infection on the controls was not very high.

EXPERIMENTAL WORK IN 1950

The project was continued in 1950, but only at Aldenham, because of the difficulties and delays caused by wind at Folkestone in 1949. The experiment was smaller and was done, as in 1948, with a hand-pressure sprayer and a long lance. The first spraying was on 28 April, and the second on 19 July. Twenty-two of the trees in the centre rows, which had been used as additional controls in 1949, were now brought into the experiment proper. The trees sprayed in 1949 were, with a few exceptions, given the same treatment in 1950. Again spraying was restricted to a single strength of DDT as miscible oil and as dispersible powder.

Information on disease activity in 1949 was available for sixty-nine of the trees used in the 1950 sprayings. Their behaviour in 1950 is shown in Table 7.

Once again there is little to suggest that the disease reappears actively following previous infection.

TABLE 7. *Recurrence of active symptoms at Aldenham in 1950*

	Treatment in 1950	No. of trees	No. showing active symptoms in 1950
Trees showing symptoms in 1949	Controls	10	6
	Sprayed	3	1
Trees not showing symptoms in 1949	Controls	18	9
	Sprayed	38	14

The results of the spraying are shown in Table 8. The assessment was made on 27 September, and on this occasion a slightly different rating was used:

0 No active disease.
1 Trace of active disease.
2 Trace or one-quarter of the crown actively diseased.
3 one-quarter to one-half of the crown actively diseased.
4 one-half to three-quarters of the crown actively diseased.
5 three-quarters to the whole of the crown actively diseased.

TABLE 8. *Effect of spraying elms at Aldenham in 1950*

	No. of trees	Rating 0	1	2	3	4	5	Total ratings	Average rating
DDT powder 1·5 %	28	15	2	3	4	2	2	38	1·36
DDT oil 1·5 %	25	19	5	1	—	—	—	7	0·28
Total sprayed	53	34	7	4	4	2	2	45	0·85
Controls	38	19	4	2	3	5	5	62	1·63

Again DDT in miscible oil gave the best results, whereas in dispersible powder it could hardly be considered satisfactory.

DISCUSSION

Two things emerge fairly clearly from the results; that a measure of protection against *Scolytus* attack, and consequent infection by *Ceratostomella ulmi*, can be given to elms by spraying twice during the season with an insecticide; and that DDT in miscible oil diluted to a strength of 1·5 % is suitable for this purpose. On the other hand, it is quite clear that this spraying does not normally give perfect protection, and may on occasion fail badly, the reasons for that failure (Folkestone in 1949) not being apparent.

The strength of DDT, 1·5 %, which gave reasonable control in these experiments agrees roughly with American experience. In the United States (Swingle *et al.* 1949) 2 % has been recommended, and in Canada (McCallum & Stewart, 1951) 1 %. Higher concentrations, ranging from 12·5 % when the trees were dormant to 6·25 % when they were active, have been used when the spray was applied by a mist blower (Diedrich & Balin, 1949; Plumb, 1950), but a smaller volume was used (5–8 pints per tree for trees 50–75 ft. high). These mists are said (Anon. 1948) to use less DDT, to give better coverage, but to increase the time taken. Considering that a special sprayer is required, it is unlikely that they would appreciably lower the cost of the whole operation.

The data collected on the recurrence of the disease in the trees included in the experiments in general support American (Banfield, 1947; Banfield *et al.* 1947), and British observations that the fungus has difficulty in passing from one annual ring to the next, and that therefore active appearance of the disease in a tree is usually the result of a fresh infection. If this were not so, not only would spraying be of no avail on trees already infected, but its value on uninfected trees would be lessened, as a single failure to prevent infected beetle feeding (and it is evident from the experiments that such failures may occur) would lead to permanent infection of the tree.

It must be realized that the trees included in the experiment were, for elms, small, and that efforts to treat larger trees would require even more powerful apparatus, and involve greater difficulties with drifting spray. In 1949 when the spraying was done on a contract basis the cost was £1. 2*s*. 6*d*. per tree for the two sprayings. No doubt a parks authority, for instance, owning its own spraying plant, could lower these costs. On the other hand, a smaller number of trees done by contract, or large trees, however the spraying was done, would cost more. Over the period of 50 or more years needed to produce timber, the cost of spraying would undoubtedly exceed the final timber value of the elm, and £1 a year is a heavy price to charge against amenity alone. It would appear, therefore, that this treatment is appropriate only for small elms of particularly high sentimental or amenity value.

Unless chemotherapy, which is now being developed in America, but which costs about the same as DDT spraying, can be cheapened, the control of the disease must still rest with the older methods, general sanitation to reduce the population of infected beetles, combined with efforts to breed or select resistant elms.

Thanks are due to the Forestry Commission under whose auspices the work was carried out, to Imperial Chemical Industries, who supplied the materials for the experiment in 1948, to my colleagues in America for their encouragement and advice, and particularly to Mr H. L. Loughborough, who superintended the spraying arrangements throughout.

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THE CONTROL OF BLOWFLIES INFESTING SLAUGHTER-HOUSES

III. LARGE-SCALE EXPERIMENTS AT A DOMESTIC-REFUSE DEPOT

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(With Plate 9 and 1 Text-figure)

A field experiment has been conducted to control blowflies which fell as mature larvae from railway wagons carrying domestic refuse and infested the soil at the sidings of a refuse collecting and sorting depot.

The surface of the soil was treated so that the insects were exposed to insecticide both as migrating larvae and newly emerged adults. Dusts and emulsions of DDT and BHC were compared on the basis of equal cost. A method was evolved for obtaining quantitative measurements of the effectiveness of the treatments by trapping flies emerging from the soil.

DDT was more effective than BHC, dusts were more effective than emulsions, and small quantities applied twice weekly were more effective than a correspondingly heavy dose applied monthly. DDT dust applied twice weekly was estimated to have killed 79·7 % of larvae and pupae and 95·7 % of emerging adults: overall control was 99·1 %. Equally high control was maintained when the method was adopted as a routine measure by the local authority.

INTRODUCTION

In earlier work, Green (1953) found that, although blowflies were prevented from breeding at a large slaughter-house, considerable numbers were attracted from breeding places beyond the premises. The investigation was, therefore, extended during 1951 to cover other breeding grounds, and this paper describes some extensive trials at a heavily infested refuse sorting and disposal depot situated about three-quarters of a mile north-east of the slaughter-house.

The depot and associated railway sidings covered an area of about 400,000 sq.ft. and received 400–700 tons of domestic refuse daily from four London boroughs. Infestation spread over about 60,000 sq.ft., of which some 15,000 sq.ft. was very heavily infested. Collecting vehicles assembled in a large concrete yard and discharged their loads into hoppers from which conveyer bands took the refuse through an enclosed sorting plant where salvageable material was removed. The discarded refuse was then fed into metal railway wagons, each with a capacity of about 20 tons, which were shunted into the adjoining sidings to await despatch to a rural tip 15–24 hr. later.

During the summer months, vast infestations of migrating blowfly larvae occurred in the ballast of the railway tracks, and the adults which subsequently emerged spread over the surrounding district causing considerable concern to the local health authorities. After an initial survey by Mr F. R. Cann of the Infestation Control Division, Ministry of Agriculture and Fisheries, arrangements were made with the several authorities for the Pest Infestation Laboratory to investigate the problem and conduct field trials.

Nature and degree of infestation

The blowflies were a mixed population similar to that occurring at the nearby slaughter-house (Green, 1951, 1953), with *Calliphora* predominant in spring and late autumn and *Lucilia* during the summer.

The infestation was caused by larvae falling from the railway wagons during their short stay in the sidings. Examination of the refuse at the depot showed very little animal matter present. Since the larvae were almost all fully grown and the refuse was normally on the premises for less than 24 hr., the infestation must obviously have been in the domestic bins before collection of the refuse. In collaboration with Mr F. R. Cann, an examination was therefore made of some refuse bins in the district. The local authority provide separate bins for kitchen waste which is collected every 2 or 3 days and processed for pig food, but it was found that many people deposited such waste in their domestic bin where it became infested. Bins containing fish, meat, or bone were easily identified without examination because of their attendant adult blowflies: fish-heads were particularly attractive and became infested even if wrapped in paper. All domestic bins were emptied every 7 days, and the contents of seven out of ten bins examined while awaiting collection were found to contain blowfly larvae; no housefly larvae were seen. The numbers of larvae varied from about 20 to 300 per bin. Since each railway wagon holds about 20 tons of refuse (the contents of about 800 bins) and an average of 21 wagons are filled each day, it is not surprising that large infestations occurred at the depot.

Larvae fell from cracks in and around the four wooden doors on the railway wagons, sometimes at rates as high as 7200 per hr. per wagon, and burrowed into the top 2 in. of soil usually within 20 ft. of the rails. Samples of the top 2 in. of soil taken during August 1951 indicated that parts of the sidings were infested with larvae and pupae at the rate of about 1600/sq.ft. Fortunately the waste oil, water, and steam from the engines caused a high mortality of larvae, and many were eaten by the flocks of birds which inhabited the area. Trapping experiments, however, indicated that large numbers survived and reached the adult stage: during the peak emergence period, 4–7 August, flies were emerging from control plots at the average rate of 8·12/sq.ft./day or 121,800/day had the whole 15,000 sq.ft. of heavily infested ground remained untreated.

EXPERIMENTAL PROCEDURE

To be suitable for adoption on a large scale by the local authorities a treatment needs to be simple to apply, and the material should be cheap and readily available.

Treatment of the refuse, either by steam heat or by admixture of insecticide, while it was on the conveyer belt was regarded as impracticable. It was therefore decided to aim at control by treating the surface of the ground at the railway sidings with DDT or BHC. The insects would then be exposed to an insecticidal film both as migrating larvae and as newly emerged adults.

Varying degrees of success have been recorded by other workers using DDT and BHC for control during the larval and pupal stages. Almost all published work deals with treatment during the feeding phase although, using prepupae of *Lucilia cuprina*, Shanahan (1946) found that BHC emulsions prevented successful pupation, and Waterhouse (1947) found suspensions and emulsions of DDT had no contact toxicity. Lyle-Stewart (1946) and Cragg (1947) attributed some larvicidal action to DDT in sheep dips, whereas Miller (1951) concluded that DDT was ineffective against maggots of the sheep blowfly. Furman (1946) obtained good results with DDT in kerosene against flies breeding in latrines but made no observations on larval control, while Simmons & Wright (1944) and Travis & Bohart (1946), using kerosene solutions and emulsions, observed some kill of larvae and puparia. Olson & Dahms (1945) found DDT emulsion unsatisfactory but obtained good control with 10% DDT dust, whereas Tanada, Holdaway & Quisenberry (1950) reached the reverse conclusions. Hall (1948) and McDuffie, Lindquist & Madden (1946) conclude that DDT is of little value as a larvicide, but the latter found BHC gave much better results.

It was felt that an efficient coating on the surface of the ground should give good control of emerging adults and might, at the same time, kill some migrating larvae. Two formulations, dusts and emulsions, were selected for trial. Practical considerations restricted the experiment to only one type of spray, and emulsions were preferred to wettable powders which are more difficult to mix and tend to settle out and block nozzles. The dusts had shown promise in the slaughter-house investigations, were supplied ready for use, could easily and quickly be applied to large areas, and made treated areas clearly visible (an important factor if using unskilled or unenthusiastic labour); on the other hand, they were difficult to apply to flat ground during windy weather. The emulsions were recommended by the manufacturers for use on refuse infested with larvae and were not so affected by wind but required transported water, needed measuring and mixing, and were not visible after application. The DDT preparations, although nominally 5% dust and 15% emulsion, contained 4 and 12% respectively of the *p-p′*- compound; the BHC dust contained 4% of mixed isomers but 0·5% γ-isomer and the emulsion contained 20% γ-isomer.

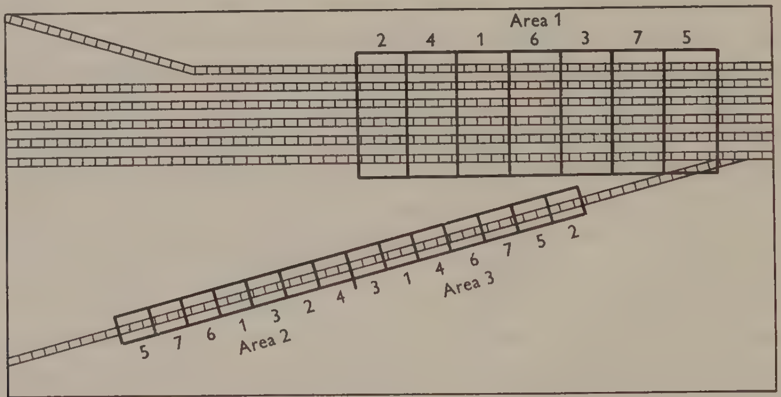
It was intended to apply all treatments with the type of apparatus likely to be used in practice but, in the work on experimental plots, the dust had to be applied by hand sieving to reduce as much as possible the amount of insecticide drifting on to other plots. The sprays were applied by a stirrup pump fitted with a lance and paired limewashing nozzles.

Basis of comparison

It was decided to compare materials on the basis of equal cost; labour costs could be estimated separately.

A monthly dosage figure was selected which gave for all insecticides a rate of application which was at least as great as the maker's recommendation. The dusts were applied both as single, monthly treatments and as twice-weekly treatments at one-eighth of the dosage, but, the emulsions were applied only once monthly because of high labour costs and the difficulty, at the recommended dilutions, of obtaining complete coverage of the ground with small doses of liquid insecticide. For each treatment the cost of materials was about 7*s.*/1000 sq.ft./4-week period. Table 1 shows in detail the rates of application.

There were three treatment areas: two, both of which measured 15 × 140 ft. were each divided into seven plots of 300 sq.ft.; the third, which measured 67 × 210 ft., was divided into seven plots of 2000 sq.ft. approximately. Each set of plots consisted of six treated and one untreated, the plan of which is shown in Text-fig. 1.



Text-fig. 1. Plan of treatments: 1, DDT dust, twice weekly; 2, DDT dust, once monthly; 3, BHC dust, twice weekly; 4, BHC dust, once monthly; 5, DDT emulsion; 6, BHC emulsion; 7, untreated.

Measurement of efficacy

A method was devised for obtaining a quantitative comparison of the efficacy of the various treatments by trapping emerging adults. Each trap (Pl. 9, figs. 1, 2) measured 6 × 6 × 6 in. and, except for a sliding glass lid, was made of sheet aluminium. A cone, 3 in. high and with an aperture $\frac{1}{4}$ in. across, was fixed $\frac{1}{2}$ in.

from the bottom of the four sides. Two pointed legs, fixed to opposite bottom corners, could be pushed into the soil so that the trap was fixed firmly to the ground. In this position, light was excluded from the bottom of the trap except for that passing through the aperture in the cone. Flies that emerged from the ground covered by the cone were attracted to this source of light and tended to enter the top part of the trap.

TABLE 1. *Insecticides and rates of application*

				Rate of application		
Insecticide	Active ingredient (%)	Dilution	Applications/4 weeks	Treatment/1000 sq.ft.	Active ingredient (mg./sq.ft./4 weeks)	Cost of material/4 weeks
DDT dust	4	—	1	7720 g. (17·04 lb.)	308·8	7s.
			8	965 g. (2·13 lb.)	308·8	
BHC dust	0·5	—	1	5223 g. (11·53 lb.)	26	
			8	653 g. (1·44 lb.)	26	
DDT emulsion	12	1:15	1	27·22 l. (5·99 gal.)	204	
BHC emulsion	20	1:69	1	12·35 l. (2·72 gal.)	38·4	

All flies that died under the cone were considered to have been killed by the treatment, whereas those that reached the top of the trap were considered to have survived the treatment, although many were affected and could not have flown away. Figures obtained, therefore, represented the minimum control achieved. Traps were emptied and moved twice weekly so that the areas sampled were exposed to normal treatment and weather conditions. Fourteen traps were used for each treatment, four on each of the 300 sq.ft. plots and six on each of the 2000 sq.ft. plots.

Trapped flies were classified as *Lucilia*, *Calliphora*, or other Diptera. The other Diptera represented only 6·4% of the emerging flies and, although they were not further classified, it was observed that they comprised mostly *Musca domestica*.

RESULTS

Control of larvae and pupae

It was expected that control would be achieved primarily by killing freshly emerged adults as they crawled over the treated ground, but a very high proportion of the insects was found to have been killed in the larval and pupal stages. Although comparative counts were not made, it was observed that with the DDT treatments a high proportion of larvae died in the soil, whereas with the BHC treatments the majority of dead larvae were on the surface of the ground. With both insecticides many larvae which pupated failed to emerge. There was no evidence that adults died in the ground and, for the purposes of calculation, it was assumed that control of adults occurred only after emergence from the soil.

The degree of control obtained in the larval and pupal stages is illustrated in Table 2, where the total numbers of flies trapped are compared for each treatment and the degree of control is expressed in relation to the numbers emerging on the untreated plots.

TABLE 2. *Comparison of adult emergence to illustrate control at larval and pupal stages*

		Lucilia		*Calliphora*		Other Diptera		Total	
Insecticide	Applications/4 weeks	No. emerging	Control (%)	No. emerging	Control (%)	No. emerging	Control (%)	No. emerging	Control (%)
DDT dust	1	105	77·8	178	64·0	12	81·8	295	71·5
	8	67	85·9	135	72·7	8	87·9	210	79·7
BHC dust	1	140	70·5	364	28·5	13	80·3	517	50·0
	8	142	70·0	288	41·8	18	72·7	448	56·7
DDT emulsion	1	188	60·3	228	53·9	19	71·2	435	57·9
BHC emulsion	1	285	39·9	504	0	28	57·6	817	21·1
Untreated	—	474	—	495	—	66	—	1,035	—

In all instances DDT gave better control than the corresponding BHC treatments. Dusts were more effective than emulsions, and frequent applications of small quantities of dust were slightly more effective than heavy applications at longer intervals. Better control of *Lucilia* was obtained than of *Calliphora* and better control of other Diptera than of blowflies.

Control of adults

Control at the adult stage, although probably due largely to the action on the adults of the residual surface film, may well have been enhanced by insecticide absorbed during the larval stage: Bruce & Decker (1950) observed a high rate of mortality among adult flies which were reared from larvae previously exposed to DDT. The figures, summarized in Table 3, illustrate the comparative toxicity of the various treatments to those adults which successfully emerged from the soil. The numbers of flies dying under the cones are expressed as percentages of numbers emerging and recorded in Table 2.

TABLE 3. *Comparative toxicity of the treatments to emerging adults*

		Kill (%)			
Insecticide	Applications/4 weeks	*Lucilia*	*Calliphora*	Other Diptera	Total
DDT dust	1	84·8	89·3	41·7	85·8
	8	91·0	97·8	100	95·7
BHC dust	1	79·3	81·0	46·2	79·7
	8	92·3	90·3	83·3	90·6
DDT emulsion	1	59·0	79·4	36·8	68·7
BHC emulsion	1	74·4	60·1	53·6	63·6
Untreated	—	11·4	12·5	4·5	10·5

The kills recorded on the untreated plots practically all occurred on the 2000 sq.ft. plot, which adjoined a BHC-dust treatment, and almost certainly resulted from dust that, when applications were followed by dry, windy weather, could be seen blowing from the treated plot.

Again, DDT was more effective than BHC, dusts were more effective than emulsions, and frequent applications of small quantities of dust were more effective than heavy applications at longer intervals: differences were, however, less. In general, treatments were about equally effective against *Lucilia* and *Calliphora* but poorer control of other Diptera was obtained.

Overall control

The total effect of the various treatments has been assessed in Table 4 by relating the numbers of surviving adults on the treated plots, i.e. those which reached the top of the traps, to the numbers emerging on the untreated plots. All flies trapped on the untreated plots were included as normal emergence, it being assumed that the few deaths which occurred were caused by drifting insecticidal dust.

TABLE 4. *A comparison of the overall effect of treatments on the emergence of adult flies*

		Control (%)			
Insecticide	Applications/4 weeks	*Lucilia*	*Calliphora*	Other Diptera	Total
DDT dust	1	96·6	96·2	89·4	95·9
	8	98·7	99·4	100	99·1
BHC dust	1	93·9	86·1	89·4	89·9
	8	97·7	94·3	95·5	95·9
DDT emulsion	1	83·8	90·5	81·8	86·9
BHC emulsion	1	82·5	59·4	80·3	71·3

DISCUSSION

Effect of rainfall

Rain seemed likely to reduce the effectiveness of the surface films, but treatments remained equally effective regardless of rainfall which varied from 0·15 to 2·58 in. per 4-week experimental period.

Effect of the insecticides on birds

Large numbers of larvae were eaten by the birds, mostly sparrows, which inhabited the area and which, if no larvae were available on the ground, dug beneath the surface to find them. These birds appeared to feed indiscriminately on both treated and untreated plots and almost certainly ate many larvae with adhering and absorbed insecticide. After 3 months' treatment several birds appeared to be poisoned to varying degrees. Many developed a staggering walk and irregular flight and three were noted which had lost all co-ordination of limbs.

General remarks

A surprising feature of the experiment was the high kill of larvae, especially with the DDT dust. The larval control recorded by previous workers has been of a relatively low order but, as pointed out earlier, experiments were usually concerned with feeding larvae. It may be that the migratory larva is especially susceptible. Waterhouse (1947) showed that young prepupae of *Lucilia cuprina* were more easily killed by DDT dust than older ones, and was inclined to the view that the larval cuticle changes in permeability as pupation approaches but found, nevertheless, that long exposures were necessary.

DDT showed better control than the corresponding BHC treatments, although the greater numbers of dead larvae on the surface of the ground often gave a visual impression that BHC was the more effective.

Dusts were clearly more effective than emulsions, the difference probably being greater than the figures indicate because drifting insecticidal dust, such as caused deaths on the untreated plots, may well have caused some deaths on the sprayed plots; there was, however, no way of determining this.

Highest kills were obtained when the dust was applied in small quantities twice weekly rather than as a heavy dose once monthly and, although with DDT the difference in kill was only 3·2%, it must be borne in mind that, during the peak period, this figure represented an emergence at the sidings of 3000–4000 blowflies per day. Under the conditions described, 99·1% was considered to be excellent control, and it is probable that the final kill was even higher since almost all adults which were classed as surviving the DDT dust treatment were badly affected. Olson & Dahms (1945) found that all adults died within 2 hr. of emergence from material treated with DDT dust, and Furman (1946) found that the majority of flies died within 24 hr. of emergence from latrine pits treated with DDT in kerosene.

The twice-weekly application of 5% DDT dust was therefore recommended for adoption by the local authorities concerned.

PRACTICAL APPLICATION

During the summer of 1952, employees of the local authority applied 5% DDT dust twice weekly to all heavily infested parts of the sidings; nearby vegetation and other parts of the sidings were dusted less frequently but at least once each month.

Laboratory staff acted only in an advisory capacity, but made regular observations and carried out some fly trapping. Many dead larvae were seen, and the results of trapping indicated that the degree of control achieved in practice was at least as good as that obtained under experimental conditions.

At the end of the second season of heavy and widespread use of DDT there was no indication of increased resistance of blowflies.

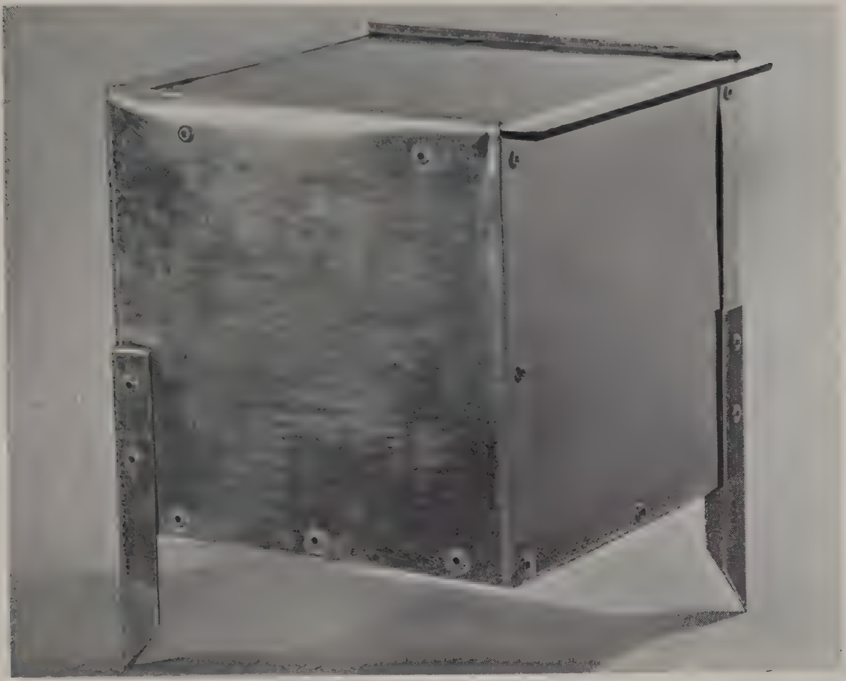


Fig. 1.

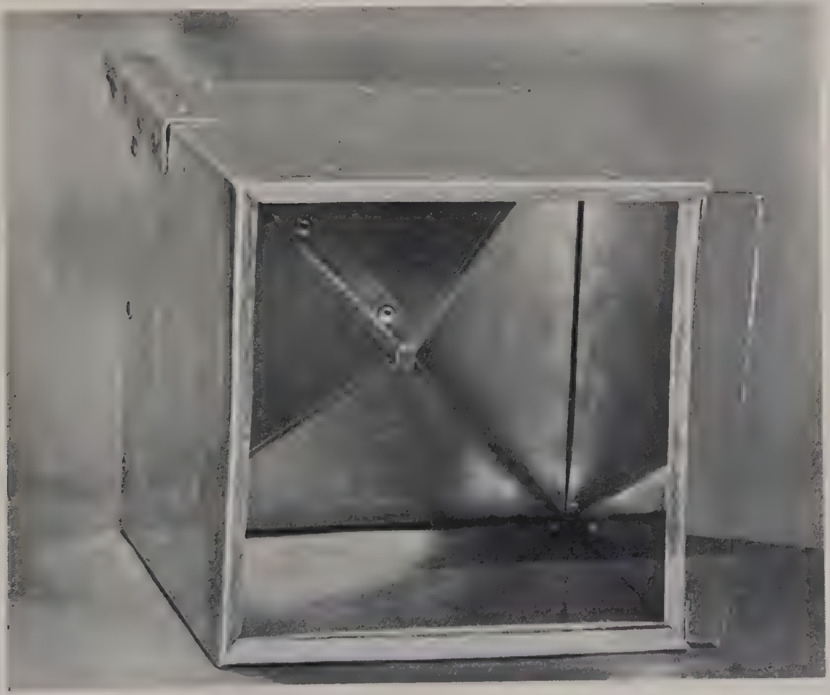


Fig. 2.

Green & Kane—*The control of blowflies infesting slaughter-houses*

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EXPLANATION OF PLATE 9

Figs. 1 and 2. Side and top views of apparatus for trapping flies emerging from the ground.

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THE DIFFERENT DISTRIBUTION OF TWO BRASSICA VIRUSES IN THE PLANT AND ITS INFLUENCE ON SPREAD IN THE FIELD

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Previous knowledge provided no explanation for the greater prevalence of cauliflower mosaic than of cabbage black ring spot in field crops of cauliflower. Both viruses are spread principally by *Myzus persicae* and *Brevicoryne brassicae*, and both are transmitted equally readily from infected seedlings. Cabbage black ring spot virus has a much wider host range, and sap from infected leaves has a higher dilution end-point than sap from leaves infected with cauliflower mosaic virus.

At least part of the difference between the rate at which the two viruses spread in the field may be accounted for by the different manner in which they are distributed in old infected plants, and the effect this has on transmission by aphids. Cauliflower mosaic virus occurs in high concentration in all the new leaves produced by infected plants. Cabbage black ring spot virus, on the other hand, occurs mainly in the older leaves, and even there is localized in parts that show symptoms. Only in recently infected plants does cabbage black ring spot virus occur in young leaves.

After flying, most aphids alight on the upper parts of plants; they are therefore less likely to acquire cabbage black ring spot virus than cauliflower mosaic virus. It may be significant that cabbage, a host in which old leaves are in a more favourable position for alighting aphids than are those of cauliflower, is also often extensively infected with cabbage black ring spot virus.

Cauliflower mosaic virus (ClMV) is the most widespread virus affecting cultivated cruciferous crops; most records are of this virus alone, although it has been described as occurring in conjunction with cabbage black ring spot virus (CBRSV) in cabbage and cauliflower crops (Walker, LeBeau & Pound, 1945; Kvicala, 1948). During surveys of the principal winter cauliflower-growing areas of England in 1950 and 1951, cauliflower mosaic was found frequently, but cabbage black ring spot only rarely; also where CBRSV occurred only few plants were infected, whereas many were infected with ClMV. In 1952, however, CBRSV was found in all the areas visited, though infections were fewer than with ClMV; plants infected with both viruses were common.

CBRSV has a much wider host range than ClMV, which is confined to cruciferous plants; also sap from leaves infected with CBRSV has a dilution end-point of about 1 in 10,000, in contrast to 1 in 1000 for sap from plants infected with ClMV (*ibid.*). These facts suggest nothing to explain the greater prevalence of cauliflower mosaic, and it was thought that the most likely explanation would be found by studying the virus vectors. Kvicala (1948) found that *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (L.), the two species of aphids that usually colonize brassica plants, transmit both viruses, whereas *Myzus ornatus* Laing transmits ClMV only. Tests at Rothamsted have shown that several aphid species transmit ClMV more readily than CBRSV, but *Aphis fabae* (Scop.) transmits CBRSV more readily than ClMV. Eighteen only of the 400 to 500 species of British aphids have been tested, so it is premature to conclude that most non-colonizing aphids are better vectors of ClMV than of CBRSV. Non-colonizing aphids may sometimes carry the viruses, but the results of field experiments suggest that *Myzus persicae* and *Brevicoryne brassicae* are the only common vectors. These two species transmitted CBRSV no less readily than ClMV when aphids, previously prevented from feeding for 2 hr., were allowed to feed for 5 min. on leaves infected with both viruses before they were transferred to young cauliflower or turnip plants; of 117 plants colonized at different times, nineteen became infected with both viruses, six with ClMV only, and twenty-nine with CBRSV only. Thus differences in the rate of spread of ClMV and CBRSV cannot be accounted for by differences in the relative efficiency of vectors.

Concentration of virus in plant sap

The temperature at which plants are grown greatly affects the concentration of both viruses in cabbage plant sap (Pound & Walker, 1945). Plants growing at 28° C. contain more CBRSV than plants at 16° C., whereas the opposite is true for ClMV. In mid-western U.S.A. symptoms of cabbage black ring spot appear in early summer when temperatures are high, remain severe until autumn, when they decrease and cauliflower mosaic becomes more evident. In Britain, however, when both viruses occur in crops, the symptoms caused by CBRSV are not predominant in summer in cauliflower, though they may be in cabbage, in which symptoms caused by ClMV are usually indistinct. This is probably because high temperatures are rare in Britain. Mean summer temperatures in the open are usually between 12 and 17° C., insufficient to mask symptoms in cauliflowers infected with ClMV, though masking is common under glass. It may be thought that the low summer temperatures in Britain cause plants to have too little CBRSV to be easily acquired by vectors, but experiments described below show that under certain circumstances CBRSV can spread as readily as ClMV. It seems unlikely that temperature has much effect on the availability to aphids of either virus in Britain.

Sap from young plants infected with CBRSV caused about nine times as many local lesions on tobacco as did sap from mature plants showing severe symptoms. The results of two experiments suggest that the virus increases in concentration for some weeks after infection and then decreases as the plant ages. Whether the virus content of infected sap is related to the ease with which aphids become infective is doubtful, for aphids fed on infected seedlings 2 weeks after infection caused four times as many lesions on tobacco as aphids fed on older plants, although sap from the older plants caused more lesions on tobacco than sap from the seedlings.

Thus aphids may be able to obtain CBRSV more readily from young than from old infected plants. Aphids were able to transmit ClMV as readily from old as from young infected plants in field experiments on spread from single infected plants of different ages; the results shown in Table 1 were obtained.

Table 1. *Mean numbers of new ClMV infections in plants surrounding single 'infectors'*

Exp.	The same age as the crop	Old plants
1	5	6
2	14	23
3	15	13

The influence of one virus on the other in mixed infections

Cabbage black ring spot usually shows on cauliflower as small blisters (slightly raised circular areas), sometimes distinguishable only because they lack waxy 'bloom'; on older leaves the blisters often change to chlorotic or necrotic blotches or rings. Cauliflower mosaic usually shows as vein-clearing on the first few leaves that have symptoms, and as well-defined vein-banding in later stages of infection. When both viruses are present together these typical symptoms are superseded by a general mottle, and the appearance of the plant suggests that ClMV is dominant (Broadbent & Tinsley, 1953). Kvicala (1948) stated that ClMV suppressed CBRSV in cauliflower; his evidence was the reduction in number of CBRSV lesions on tobacco between two tests, one from an early stage of a mixed infection, and a second from the same plant 8 weeks later. The reduction more likely occurred because the plant aged. The aphid-transmission tests reported above, in which CBRSV was transmitted more often than ClMV from mixed sources, showed that CBRSV is not suppressed by ClMV.

The persistence of CBRSV in the presence of ClMV was confirmed by tests with cauliflower, variety Extra Early Roscoff, infected as seedlings with mild and severe strains of CBRSV, alone and together with mild and severe strains of ClMV. Sap from one plant of each treatment was inoculated to tobacco on seven occasions (24 March, 4, 7, 30 April, 16 May, 27 June, 8 August); the sap was obtained separately from: (*a*) the growing point + 1–2 leaves (young); (*b*) the next 2–4 leaves (middle); (*c*) the remaining 3–5 leaves (old). Two plants of each treatment were planted outside (17 April) under insect-proof nylon cages, and were used for the last two series of inoculations, the last being after the plants had curded. The infectivity tests were made by the half-leaf method on tobacco; only the three youngest leaves were used, because older tobacco leaves resist infection.

Fewer lesions were obtained with the last four than with the first three series of inoculations; the reduction may have been caused partly by the tobacco plants increasing in resistance with change from spring to summer. This is unlikely, however, for the second and third series gave nine times as many lesions as the sixth and seventh, which is the same ratio as that obtained when sap from young and old CBRSV-infected plants was inoculated on the same day. Neither could the reduction be attributed entirely to temperature differences, because the fourth and fifth series yielded fewer lesions than the second and third, although the temperature in the glasshouse was higher for the later series. The reduction was most probably caused by the plants ageing.

The total numbers of lesions produced by the two strains of CBRSV were very similar (1586 for the severe strain and 1578 for the mild strain), showing that the severity of symptoms in cauliflower is not necessarily correlated with virus concentration. Neither strain of ClMV significantly affected the concentration of CBRSV. Sap from old leaves was most infectious; the ratio of lesions produced by sap from young, middle and old leaves was 1:10·7:37·8.

Distribution of virus in the plant

The small concentration of CBRSV in the young leaves suggests a reason for its failure to spread as rapidly as ClMV in field crops; cauliflower mosaic symptoms occur on most leaves of field plants, however long they have been infected, whereas cabbage black ring spot symptoms are usually obvious only on the oldest leaves of plants infected for some weeks. Glasshouse tests showed that both viruses are distributed in much the same way in newly infected plants, although CBRSV was localized in the leaf. Within 1 or 2 weeks of inoculating a lower leaf, the viruses invaded young leaves around the growing point; but they usually failed to enter mature leaves above or below the inoculated leaf. Cauliflower mosaic symptoms developed in many leaves that were partially expanded when the virus reached the growing point, and in all leaves subsequently developed. Cabbage black ring spot symptoms developed in partially expanded leaves, and in the first few leaves produced after infection, but as the plant grew, the new leaves were symptomless and only middle and lower leaves showed lesions.

To find whether or not symptoms reflect the distribution of CBRSV, sap from leaves of different ages on infected field plants was inoculated to tobacco plants (Table 2). The results showed that virus concentration was very low in young leaves. As a further test, sap from every leaf of an Extra Early Roscoff cauliflower, grown under glass, was inoculated to tobacco 7 weeks after the plant had been infected with CBRSV, with the results shown in Table 3.

Many substances inhibit infection with viruses, and although the results on tobacco strongly suggested differences in virus concentration, they could have occurred because of virus-inhibition occurring in different quantities in leaves of different ages.

Crude sap from *Nicotiana glutinosa* infected with CBRSV was mixed with sap from either young or old leaves of uninfected cauliflower or with distilled water, in the proportion of two parts CBRSV sap to one of diluent: six half-leaves of

TABLE 2. *Numbers of lesions on tobacco inoculated with sap from young, medium and old leaves of various brassica plants infected with CBRSV*

Variety	Numbers of lesions from leaves		
	Young	Medium	Old
Cauliflower			
St George	—	29	873
Seale Hayne 4DK7	—	0	778
Midsummer	—	24	1779
Champion	—	19	1806
Extra Early Roscoff	0	30	134
Extra Early Roscoff	2	19	632
Extra Early Roscoff	1	17	135
Brussels sprout			
Cambridge 1	0	—	717
Cambridge 1	0	—	597
Cambridge Special	0	28	30
Cambridge Special	0	13	57
Rous Lench	0	11	162
Rous Lench	0	1	29
Ashwell's strain	0	5	10
Ashwell's strain	0	0	64
Kale (Marrowstem)	0	—	229
Kale (Marrowstem)	0	—	15

TABLE 3. *CBRSV symptoms on leaves of a cauliflower plant, and numbers of lesions on tobacco inoculated with sap from them*

Leaf	Symptoms	Total lesions
1 (oldest mature leaf above inoculated leaf)	None	0
2	Very many blisters	955
3	Many blisters	959
4	Many blisters	751
5	Few blisters	419
6	None	29
7	None	40
8	None	8
9 (youngest)	None	0

tobacco were rubbed with each inoculum. Numbers of lesions did not differ greatly; the totals were 472 with water, 390 with sap from old leaves and 266 with sap from young cauliflower leaves. Other tests with sap from cauliflower and Brussels sprout plants gave similar results. The differences are small compared with those between lesions obtained with sap from young and old infected leaves, and it is concluded that the numbers of lesions obtained with sap from leaves of different ages reflect the distribution of CBRSV within the plant.

Aphid transmissions confirmed the inoculation results: *Myzus persicae*, after fasting for 2 hr., were fed for 5 min. on young, middle or old leaves of infected plants brought from the field in early November, and were then placed on young tobacco plants (three replicates per treatment, and five aphids per replicate). The total numbers of lesions caused by their feeding were 0 (young), 9 (middle) and 15 (old).

CBRSV is not only unequally distributed in leaves of different ages, but also within individual leaves. More virus occurs in the blisters or rings than in symptomless areas of the leaves. When equal numbers of disks were taken with a cork borer (310 in four tests) from blisters, and the extracted sap was rubbed on tobacco, 115 lesions were obtained, compared with twenty-six by sap from disks from the symptomless areas.

Distribution of alighting aphids on plants

Winged aphids often take off from, and alight on, upper parts of plants (Joyce, 1938; Müller & Unger, 1951; Broadbent, 1953). *M. persicae*, flying over a field of melon plants, made about fifty landings per minute per plant, about the same numbers leaving during this time (Dickson, Swift, Anderson & Middleton, 1949). The aphids began feeding immediately on landing, and the feeding periods averaged about 40 sec. Aphids sometimes alighted again on nearby plants, or even on the same plant from which they had taken off. Kennedy (1950) found that a small minority of *M. persicae* stayed when they alighted on peach, their winter host; most flew off again, settlement being a gradual process.

During a migration of *B. brassicae* on 26 July 1952 their arrival on cauliflower plants was observed. At 10 a.m. many aphids were landing and taking off again at frequent intervals, sometimes without feeding, sometimes after feeding for a short time. Air movement was slight and the aphids were not swept away, but were able to maintain flight near a plant for a considerable time.

Most flight was around the upper leaves of the cauliflower plants and in the two feet of air above; most aphids failed to settle for some hours, although the plants were suitable hosts. When flight had almost ceased (at 3.45 p.m.), the winged aphids on each leaf of five plants were counted. Three of these plants, including 1 and 2 in Table 4, had been grown under an aphid-proof nylon cage, which was removed at 10.45 a.m., so the aphids there had arrived during the previous 5 hr. The other two, including plant 3 in Table 4, were young plants in a nearby field, spaced 3 × 2½ ft. apart, and previously were uninfested. 90% of the aphids settled on upper or upper-middle leaves.

Spread of virus in the field

ClMV and CBRSV behave similarly in aphid-transmission tests. Kvicala (1948) showed that ClMV may persist in fasting aphids for a few hours longer than CBRSV, but neither usually survives in feeding aphids for 1 hr. Both viruses can be acquired and transmitted by fasted aphids in very short feeding periods, so that aphid movements, like those described above, are ideal for virus spread. Field experiments have shown that flying aphids cause most spread, although walking aphids can occasionally spread virus to neighbouring plants.

TABLE 4. *Counts of winged* Brevicoryne brassicae *on previously uninfested cauliflower plants immediately after a dispersal flight at Rothamsted, 26 July* 1952

Position	Leaf description	No. of aphids	
	Plant 1 (Extra Early Roscoff, previously caged)		
1, youngest	Inner heart	7	
2	Inner heart	4	
3	Outer heart	38	
4	Outer heart	22	
5	Outer heart	55	
6	Highest, vertical	114	
7	Highest, vertical	193	
8	Highest, vertical	166	
9	Middle, inclined upwards	148	
10	Middle, inclined upwards	115	
11	Middle, inclined upwards	85	
12	Middle, inclined upwards	119	
13	Middle, horizontal	80	
14	Middle, horizontal	24	
15	Middle, horizontal	47	
16	Lower, bending towards ground	15	
17	Lower, bending towards ground	22	
18	Lower, bending towards ground	6	
19	Lower, bending towards ground	14	
20	Lower, bending towards ground	7	
	Total	1281	
	Plant 2. Infected with CBRSV (Extra Early Roscoff, previously caged)		
1, youngest	Inner heart	7	No CBRSV symptoms
2	Inner heart	8	
3	Inner heart	21	
4	Outer heart	25	
5	Outer heart	53	
6	Outer heart	24	
7	Highest, vertical	135	
8	Highest, vertical	190	
9	Middle, inclined upwards	88	A few CBRSV 'blisters'
10	Middle, inclined upwards	72	
11	Middle, horizontal	22	Moderate CBRSV symptoms
12	Middle, horizontal	7	
13	Middle, horizontal	8	
14	Lower, bending towards ground	4	Severe CBRSV symptoms
15	Lower, bending towards ground	1	
16	Lower, bending towards ground	6	
17	Lower, bending towards ground	1	
	Total	672	
	Plant 3. Infected with CBRSV (Novo, in field crop)		
1, youngest	Highest, vertical	11	No CBRSV symptoms
2	Highest, vertical	37	
3	Highest, vertical	27	
4	Highest, vertical	32	
5	Middle, inclined upwards	30	
6	Middle, inclined upwards	25	A few CBRSV 'blisters'
7	Lower, horizontal or bending towards ground	2	Severe CBRSV symptoms
8	Lower, horizontal or bending towards ground	2	
9	Lower, horizontal or bending towards ground	0	
10	Lower, horizontal or bending towards ground	2	
11	Lower, horizontal or bending towards ground	1	
	Total	169	

Percentages of aphids in different zones (means for five plants)

	%
Heart	14·6
Upper leaves	46·7
Upper middle leaves	27·5
Lower middle leaves	7·6
Lower leaves	3·6

TABLE 5. *Numbers of ClMV and CBRSV infections in batches of thirty cauliflower plants exposed at intervals near sources of infection*, 1952

Period of exposure	Nos. of infected plants ClMV	CBRSV	ClMV/CBRSV
17 Apr.–15 May	0	0	0
15 May–12 June	0	0	0
12 June–10 July	6	4	1·5
10 July–24 July	18	9	2·0
24 July–14 Aug.	24	11	2·2
14 Aug.–11 Sept.	30	26	1·2
11 Sept.–23 Oct.	6	2	3·0
23 Oct.–27 Nov.	0	0	0

When an aphid alights and feeds on a plant systemically infected with ClMV, it will have the opportunity to acquire virus; ClMV is present in the curd and flowers as well as in the leaves of cauliflower. An aphid is much less likely to acquire CBRSV from an infected plant, for this virus occurs predominantly in the lower and lower-middle leaves, and most aphids visit the upper parts of the plants. In a crop, where many lower leaves are partially hidden and access to them is hindered by neighbouring plants, lower leaves are less likely to be visited than with isolated plants. Equal numbers of plants infected with ClMV and CBRSV were planted in a Majestic cauliflower crop at Rothamsted in 1952; starting with 0·2% plants infected with each virus, the incidence of cauliflower mosaic increased to 47%, of cabbage black ring spot to 4%. CBRSV would be expected to spread more rapidly in cabbage crops, which are often more extensively infected with CBRSV than cauliflower or Brussels sprout crops, because the older leaves of cabbage are often uppermost and thus more accessible to aphids.

Both viruses are present in the young leaves of newly infected plants; CBRSV is acquired more readily than ClMV by aphids, but only from localized concentrations of virus in the infected leaves. The two viruses might be expected, therefore, to spread equally readily from recently infected plants. This they did in experiments at Rothamsted, when seed-beds were surrounded at a distance of 6 ft. by cauliflower plants, spaced 4 ft. apart, alternate plants being infected with CBRSV and ClMV. The mean percentages of infected plants in three spring-planted experiments were 1·5, 4·2 and 1·6 cauliflower mosaic, compared with 2·0, 5·1 and 1·4 cabbage black ring spot; in experiments planted in mid-August, with new sources of infection, the percentages of infected plants were 30·8% cauliflower mosaic and 27·0% cabbage black ring spot.

Further evidence that CBRSV will spread more rapidly from newly infected than from old-infected plants was obtained in a trial in the same field as the seed-bed experiments. Batches of thirty young uninfected cauliflower plants were put out for periods of 4 weeks and then were removed to a glasshouse for observation until symptoms developed. While in the field they were surrounded by infected plants at a distance of 16 ft., and there were at least 500 other infected plants nearby. The infections that occurred are given in Table 5. As the infected plants grew older, the proportion of cabbage black ring spot infections in the exposed plants decreased. New seed-bed trials were sown in mid-August, and many newly infected plants were planted around them. The numbers of plants infected with ClMV and CBRSV during the next exposure were almost the same, but later there were again fewer CBRSV infections.

After two seasons with few aphids, 1950 and 1951, the principal sources of virus in spring—overwintering brassica crops—contained little CBRSV. It is possible that the sources of most of the cabbage black ring spot found in 1952 were perennial garden plants; on a large farm in the Thames valley where cauliflower mosaic was widespread, cabbage black ring spot was common only near the farmhouse garden. Flowers of infected stocks (*Matthiola incana*) and wallflowers (*Cheiranthus cheiri*) contain CBRSV, so that the virus is not restricted to the older parts of all mature plants. The chances of CBRSV spreading from garden sources will be increased considerably when winged aphids are numerous.

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THE IMPORTANCE OF THE HOST SPECIES IN DETERMINING THE ACTION OF VIRUS INHIBITORS

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Some plant species from which viruses are difficult to transmit to other species by sap inoculation contain substances that inhibit infection. The extent to which infection is inhibited by these substances depends on the species of plants to which inoculations are made and not on the identity of the virus. The inhibitors are largely ineffective in preventing infection of the species which contain them.

Infection of cucumber was less affected than any of the other species by all the inhibitors tested.

The literature records several species of plants within which viruses have been transmitted by sap inoculation from infected to healthy plants, but from which the viruses are not easily transmitted by inoculation to plants of other susceptible species. For instance, cucumber mosaic virus is sap transmissible from infected to healthy *Phytolacca decandra* plants, but only rarely has it been transmitted from this to other susceptible species such as tobacco (Allard, 1918; Doolittle & Walker, 1925; Bhargava, 1951). Cucumber mosaic virus (Bhargava, 1951) and sugar-beet mosaic virus (Hoggan, 1933) are also sap transmissible between sugar-beet plants, but not from sugar beet to tobacco, a transfer that can, however, be made by aphids. Also, dahlia mosaic virus was transmitted from infected to healthy plants of *Zinnia elegans*, but not from this species to dahlia (Brierley & Smith, 1950), and carnation mosaic virus was regularly transmitted from carnation to carnation and to Sweet William, but only rarely from carnation to bean and to tobacco (Van der Want, 1951).

Sap extracted from the leaves of *Phytolacca decandra*, sugar beet or carnation, contains substances that can act as virus inhibitors, that is, when mixed with viruses *in vitro*, the mixtures fail to infect when they are rubbed over the leaves of normally susceptible plants. Such inhibitors seemed likely to be responsible for the anomalies encountered in transmission experiments with these hosts, but, if they are, then they must be more effective in inhibiting infection of other species than of the plants from which they were extracted. The manner in which such inhibitors act is still obscure, but evidence is increasing that their action is on the inoculated plants rather than directly on the virus particles (Gupta & Price, 1952; Bawden & Freeman, 1952). There is, too, evidence that some inhibitors interfere with virus establishment more in some species of plants than in others. For instance, trypsin (Stanley, 1934) and ribonuclease (Loring, 1942) both more effectively prevent infection by tobacco mosaic virus in *Phaseolus vulgaris* than in *Nicotiana glutinosa*, and of two inhibitors produced by *Trichothecium roseum*, one more effectively inhibits infection of *Phaseolus vulgaris* than of *Nicotiana glutinosa*, whereas the other is more effective with *N. glutinosa* than with *P. vulgaris* (Bawden & Freeman, 1952).

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The experiments we describe were made to find whether the virus-inhibiting ability of substances present in the leaves of plants also depends on the species of plant that is inoculated with it. The results show that there is such a dependence, and that the substances are largely ineffective in preventing the infection of species from which the sap comes.

MATERIALS AND METHODS

The plants used were mainly those known to contain inhibitors in their sap: *Datura tatula* L., sugar beet (*Beta vulgaris* L.), *Phytolacca decandra* L., cucumber (*Cucumis sativus* L.) and dahlia (*Dahlia variabilis* Desf.). Sap was obtained by crushing the leaves with a pestle in a mortar and pressing the pulp in muslin. The polysaccharide inhibitor isolated from *Trichothecium roseum* by Bawden & Freeman (1952) was also tested. Inoculations were made to plants that give local lesions, so that the results could be expressed quantitatively. The viruses were: tomato aucuba mosaic virus in tobacco (*Nicotiana tabacum* L., var. White Burley), *N. glutinosa* L. a hybrid between *N. tabacum* and *N. glutinosa*, and *Datura tatula*; tomato bushy stunt virus in *Nicotiana glutinosa*, cucumber, of which the first two leaves were inoculated, and *Datura tatula*; cucumber mosaic virus in French bean (*Phaseolus vulgaris* L. var. Prince), sugar beet and *D. tatula*; the Rothamsted culture of tobacco necrosis virus in French bean and sugar beet. The lesions were necrotic or semi-necrotic, except those produced by tomato bushy stunt virus in *D. tatula*; these were vague chlorotic lesions but readily countable when stained with iodine (Holmes, 1931). Cucumber mosaic virus gives countable local lesions on beans only between October and March (Bhargava, 1951).

A purified preparation of tomato aucuba mosaic virus was used; inoculum of other viruses was sap from infected plants clarified by centrifugation. Tomato bushy stunt virus came from tomato, and tobacco necrosis and cucumber mosaic viruses from tobacco plants. Equal volumes of virus preparations and substances under test were mixed, and the infectivity of the mixtures compared with that of virus preparations diluted similarly in water or sap from healthy tobacco leaves, which has little, if any, virus-inhibiting action. The inoculations were made with the undiluted mixtures and with the mixtures diluted 1/10 and 1/100 in water. Eight to twelve half-leaves of a batch of uniform plants were rubbed with the mixture or dilution of the mixture, and the different inocula tested in each experiment were randomized to reduce errors arising from differences between the susceptibility of plants.

EXPERIMENTAL RESULTS

Tables 1–3 record the results of some experiments in which the inhibiting power of sugar-beet sap and sap from *Datura tatula* was tested with three viruses on three host plants. Infection with all three viruses was affected by sap from both species, but the extent to which infection was inhibited depended entirely on the species of

TABLE 1. *The effect of sugar-beet sap on the infectivity of two viruses in French bean and sugar beet*

Virus	Dilution of the mixture	French bean plant		Sugar beet plant	
		Inhibitor sugar-beet sap	Control tobacco sap	Inhibitor sugar-beet sap	Control tobacco sap
Cucumber mosaic virus	Undiluted	1*	42	266	295
	1/10	5	51	96	104
	1/100	8	18	5	8
Tobacco necrosis virus	Undiluted	8	106	22	30
	1/5	9	50	13	20
	1/25	9	34	4	7

* Average number of lesions per half leaf.

TABLE 2. *The effect of* Datura tatula *sap on the infectivity of cucumber mosaic virus in* Datura tatula, *French bean and sugar beet*

Dilution of the mixture	*D. tatula* plant		French bean plant		Sugar-beet plant	
	Inhibitor *D. tatula* sap	Control tobacco sap	Inhibitor *D. tatula* sap	Control tobacco sap	Inhibitor *D. tatula* sap	Control tobacco sap
Undiluted	171*	158	1	60	1	193
1/10	32	11	2	48	1	69
1/100	19	0	1	4	1	1

* Average number of lesions per half leaf.

TABLE 3. *The inhibition of infection by sap from* Datura tatula *and sugar beet in* D. tatula *and sugar beet*

Virus	Dilution of the mixture	*Datura tatula* plant			Sugar beet plant		
		Sap inhibitor			Sap inhibitor		
		D. tatula	Sugar beet	Water Control	*D. tatula*	Sugar beet	Water control
Cucumber mosaic virus	Undiluted	18*	0	17	2	55	81
	1/10	14	0	12	8	32	38
	1/100	2	1	3	3	1	5
Tomato bushy stunt virus	Undiluted	43	2	66	—	—	—
	1/10	37	2	38	—	—	—
	1/100	19	7	14	—	—	—

* Average number of lesions per half leaf.

plant that was inoculated. Sap from both species greatly decreased the numbers of lesions produced by French bean, that from *D. tatula* did so in sugar beet but not in *D. tatula*, and sap from sugar beet did so in *D. tatula* but not in sugar beet. Inhibition of infectivity is not proportional to the amounts in which viruses and inhibitors are mixed, but, as the virus concentration increases, proportionally less inhibitor is needed to neutralize all infectivity (Kassanis & Kleczkowski, 1948). Hence, when concentrated mixtures of inhibitors and viruses are diluted, their infectivity, relative to that of comparably diluted control virus preparations, increases. This phenomenon is illustrated by the tests with French bean plants, with sugar-beet sap on *D. tatula* and sap of *D. tatula* on sugar beet. By contrast, when mixtures of viruses with sugar-beet sap were inoculated at various dilutions to sugar beet, or mixtures with sap from *D. tatula* were inoculated to *D. tatula*, the number of lesions produced at different dilutions agreed closely with that of the control virus preparations.

Table 4 provides further evidence of the extent to which the species of the inoculated plant can determine the infectivity of virus preparations that contain inhibitors. The viruses were mixed with sap from four different plants and with the polysaccharide from *Trichothecium roseum*, and the infectivity of the mixtures was compared with comparable virus solutions in water on five different species of plants. No inhibitor was equally effective in preventing infection of all the species, and all were less effective in preventing infection of cucumber than of the other hosts inoculated. The polysaccharide from *Trichothecium roseum* was less effective with tobacco (*Nicotiana tabacum*) than with *N. glutinosa*. In another experiment with tomato aucuba mosaic virus, the polysaccharide was tested on these two species and a hybrid between them. The average number of lesions per half-leaf produced by the mixture on tobacco was 44, on *N. glutinosa* 6 and on the hybrid 7, compared with 49, 39 and 30 produced on the opposite half-leaves inoculated with solutions

TABLE 4. *The relative effect of five inhibitors in five different species of plants*

	Tomato bushy stunt virus		Tomato aucuba mosaic virus (20 mg./l.)		
Inhibitor*	*N. glutinosa*	Cucumber	*N. glutinosa*	Tobacco	*D. tatula*
Polysaccharide from *T. roseum* (100 mg./l.)	10†	44	4	22	5
P. decandra sap, diluted $\frac{1}{50}$	10	60	4	2	5
Sugar-beet sap, diluted $\frac{1}{8}$	9	13	0	2	5
Cucumber plant sap, diluted $\frac{1}{5}$	37	83	50	22	53
Dahlia sap, diluted $\frac{1}{5}$	47	75	42	33	84
Water control	100 (89)	100 (139)	100 (24)	100 (45)	100 (19)

* Inhibitor and virus were mixed in equal volumes.

† Number of lesions per half-leaf as percentage of the number produced by the control, for which the actual number is given in brackets.

of the same virus concentration in water. Along with the ability to localize infection with tobacco mosaic virus, the hybrid has evidently also inherited from *N. glutinosa* its sensitivity to the inhibitor from *Trichothecium roseum*.

All the results are compatible with the view that inhibitors work by influencing the physiology of the host plant so that introduced virus particles do not become established and multiply or do not multiply sufficiently to form visible lesions. The extent to which infection is inhibited possibly reflects the extent to which the cellular metabolism is affected by the active substances, and it is reasonable to expect greatest disturbance when foreign substances are introduced, and that substances will cause little or no physiological disturbances when inoculated to species from which they were extracted.

Of plants we used, cucumber was more readily infected by mixtures containing viruses and any of the inhibitors tested than were any of the other test plants. We have no certain interpretation of this, but it is of interest that the sap of cucumber plants is more alkaline than that of the other test plants (Holden, 1948). Some of the most potent inhibitors, such as ribonuclease or the glycoprotein from *Phytolacca decandra*, combine with viruses *in vitro* and, though there is no evidence that the combination causes inhibition, it may block groups that need to combine with receptors in the host as a prelude to virus multiplication. Adding salts or changing pH of the mixture from 6 to 9 dissociates virus from inhibitor and increases the infectivity of mixtures when they are tested on susceptible plants (Kleczkowski, 1946). If the pH is also high in living cells of cucumber leaves, this might lead to some dissociation of virus and inhibitors. However, this is an inadequate explanation, for some inhibitors, such as the polysaccharide from *Trichothecium roseum* (Bawden & Freeman, 1952), do not combine with tobacco mosaic virus and it also fails to account for the different effects of inhibitors on different hosts having saps of similar pH values. Whatever is the cause of the greater susceptibility of cucumber to infection when inoculated with mixtures of viruses and inhibitors, this plant is clearly to be recommended when searching for alternative hosts for viruses which are difficult to transmit mechanically. Indeed, cherry ring spot virus (Moore, Boyle & Keitt, 1948) and rose mosaic virus (Fulton, 1952) have both been mechanically transmitted to cucumber plants from cherry and rose trees, which themselves have not been mechanically infected. This, at first sight, might seem to conflict with our results, which suggest that plants are not affected by inhibitors they contain, but difficulties in transmitting viruses by inoculation do not arise solely because of virus inhibitors.

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GROUNDNUT ROSETTE DISEASE IN TANGANYIKA

I. FIELD STUDIES

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(With 5 Text-figures)

Rosette disease of groundnuts in Tanganyika is brought into the crop by infective alatae of *Aphis craccivora*: spread within the crop is by apterae and alatae. During the dry season the aphids maintain themselves on self-set groundnuts and on two genera of Leguminosae: *Vigna* and *Millettia* or *Lonchocarpus*. No native source of the virus causing rosette disease has been discovered, but self-set groundnuts carry over the virus from one cropping season to the next. Syrphid larvae and other predators are important in controlling the vector. Preliminary spraying trials with 0·5% schradan gave promising results in controlling the aphids on groundnut crops and consequently checking the spread of rosette disease. Selections of the variety Mwitunde showed the lowest incidence of rosette infection and gave the highest yields in trials in 1952.

INTRODUCTION

Until the season 1950–1 groundnuts were grown only on a small acreage in the area being developed by the Overseas Food Corporation in the Southern Province of Tanganyika. During the three previous seasons rosette disease had been serious only on late-planted groundnuts in experiments. Native plantations at Masasi, about 30 miles to the south-west, however, showed a fairly high incidence, varying from 250 to 3250 rosetted plants per acre in 1948. It was clear that rosette disease might be a cause of serious loss, but continuous local study was not possible until 1950–1, when some experiments and surveys were made on the 3000 acres planted in the region.

Diseased plants of the variety Natal Common were first found in late December 1950 among self-set plants on some abandoned land. Very few of the plants, healthy or diseased, carried the vector, *Aphis craccivora* Koch, but some had flourishing colonies. This observation showed that, at least on land at the bottom of a slope, conditions permitted the vector to survive during the dry season with the subsequent threat of an early flight of vectors to newly germinated crops. On 15 January rosetted plants were found amongst seedlings that had appeared following early rain.

Rosette can spread very rapidly. In one crop, 2700 plants out of 26,200 per acre were infested with aphids on 16 January, when no rosette was observed. Rosetted plants were found on 22 January and counts at weekly intervals showed a tenfold increase in a fortnight; by late February 65 % of the plants were infected. During the three surveys the rosetted plants were classified according to whether no aphids were found, apterae only or both apterae and alatae.

TABLE 1. *Classification of rosetted plants according to type of infestation*

	Per acre		
	No aphids	Apterae only	Apterae and alatae
24 January	8	19	6
31 January	50	68	13
7 February	272	88	7

SPREAD OF THE DISEASE

Rosette is brought into a field by infective alatae. These alatae move from plant to plant depositing a few nymphs on each. Very young plants, marked as bearing an alate adult, were rarely found to carry it 48 hr. later. Also, such plants bear groups of two to seven nymphs and less than a quarter of these groups are found with the parent alata. These small colonies may increase in number until the plant is well infested.

Further spread occurs by two methods: alatae may be produced and fly freely in the crop or adult apterae may colonize adjacent plants. Fig. 1 shows the distribution of adult apterae and nymphs along the row containing a source plant and the adjacent rows. Plants near the parent colony carried small colonies headed by adult apterae, infested plants further away had, generally, one aptera and a few nymphs and three plants bore only one apterous adult. In one case of sparsely distributed self-set plants with very few infested plants, spread by adult apterae was traced as far as 5 yd. from the source plant.

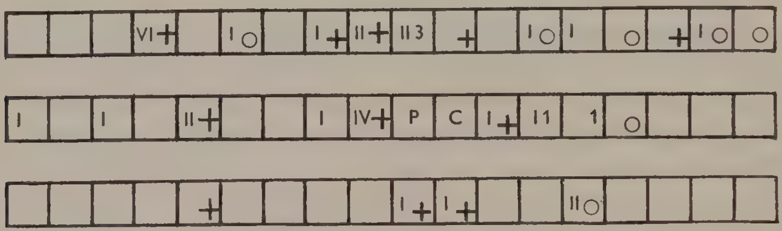

Fig. 1. The distribution of aphids on plants surrounding a parent colony P. Roman numerals, number of adult apterae. Arabic numerals, number of nymphs. *C*, small colony of adults and nymphs; +, small colony of nymphs; ○, few nymphs.

This wandering of adult apterae produces the typical patchiness that is termed secondary spread. Rosette disease causes great loss in yield, not so much because of primary infections caused by invading alatae, except when young plantings are adjacent to older infected plantings, but because of this secondary spread. The infection of several thousand plants in groups of six or more seriously affects yield, as neighbouring uninfected plants cannot compensate fully for the loss.

The amount of secondary spread depends chiefly on the presence of predators, on weather and on the variety of groundnut. Not all aphids leaving infected plants are infective, and often there is little spread, but in one variety trial three examples of much secondary spread were observed, 78, 100 and 142 plants being infected from one source.

Carry-over of the disease from one season to the next is as important as the spread during one season. After harvest seeds are inevitably left in the ground; if the soil is moist and the variety grown belongs to the short-season group, then such seeds will germinate soon after harvest or even before. Long-season varieties behave differently and their seeds have a dormant period of several weeks. Under local conditions the soil is too dry for germination by the time dormancy is broken and the seed germinates only in the early rains.

Most of the groundnuts grown during 1951 were Natal Common, a short-season type, and many nuts germinated in May. The numbers of self-set plants varied from 2000 to 95,000 per acre. Fields harvested early had higher populations than fields harvested late; and fields that were ploughed soon after harvest higher populations than fields not ploughed.

TABLE 2. *Mean number of self-set plants in thousands per acre June* 1951

	Ploughed	Not ploughed
Harvested early	56·2	39·4
Harvested late	41·3	16·1

This flush of new growth provided an excellent source of food for the groundnut aphid. The number of self-set plants, healthy and rosetted, was estimated by counting the number present in eleven square-yard samples on several adjacent strips. Infested plants, aphids and predators (eggs and larvae of syrphids and coccinellids together with adult coccinellids) were estimated on five samples of ten plants per strip. Aphids could be counted quite accurately by means of a × 10 lens up to about 100 per plant; larger numbers were estimated in groups of ten.

The results for one field are given in Table 3.

The number of self-set plants remained constant until the end of June, and then declined rapidly during July and August and more slowly from then until the field was ploughed in October. Self-set plants infested with aphids rose to a maximum during June, when 80% were infested, and then declined more rapidly than total self-set plants, to a low but still dangerous level during September and October. Most aphids occurred at the end of June when there were approximately fifty per infested plant. Winged forms reached a maximum at this period, when predators were also at a maximum. The predators rapidly reduced the numbers of aphids during the next 2 months and themselves decreased after July. Although the predators had reduced the total numbers of aphids considerably by July, the average number of aphids per infested plant was still high, about sixty, during September and October. However, the proportion of self-set plants infested was lower, 20% in September–October, compared with 80% in June. Thus both predators and the reduction in numbers of self-set plants contributed to reducing the aphid population per acre. But the predators did not exterminate the aphids and seemed to reach a balance with them.

Table 3. *Changes in numbers of self-set plants, aphids and predators during the dry season, thousands/acre*

Date ...	21. v.	9. vi.	30. vi.	26. vii.	27. viii.	3. ix.	12. ix.	22. ix.	10. x.
Self-set plants:									
Total	56·8	50·2	50·2	38·5	15·0	12·3	9·2	12·8	8·4
Infested	15·8	42·1	41·1	16·3	3·4	5·2	3·9	3·1	1·5
Rosetted	0·0	4·0	18·0	31·2	12·1	10·6	7·9	11·0	7·5
Aphids alatae	5·6	13·4	16·7	1·9	1·0	5·7	2·7	2·8	1·8
Aphids total	223	2103	1881	501	103	359	220	187	92
Nymphal alatae	0	0	424	7	14	47	24	14	16
Syrphidae	2·3	63·5	48·5	19·2	0·2	2·2	6·4	4·6	3·3
Coccinellidae	0·0	0·0	5·0	2·4	2·0	1·2	0·5	1·0	0·7

The numbers of rosetted self-set plants reached a maximum of 31,000 plants per acre in July or 80% of the total. The numbers of rosetted plants decreased considerably during the dry season but the proportion rose steadily to 90%.

When a portion of the field under observation was ploughed on 11 October, strips of self-set plants were left where the ploughs swerved away from adjacent runs, and by 5 November only an occasional plant was infested. This further reduction of infested plants may have been brought about by the adult population of predators from the area laying their eggs on the few remaining infested plants. This portion of the field was ploughed again in preparation for maize after the first rains which fell on 5 November and the remaining self-set plants were eradicated.

In another portion of the field which had been conserved by leaving grass strips, contour banks were built and a flush of early-rainy-season self-set plants appeared in the ditches and on the banks. These self-set plants were infested from the few adjacent dry-season self-set plants and high populations of aphids were quickly built up, and many of the plants became rosetted. This was observed on several fields. Both alatae and adult apterae took part in the initial movement; the further stage of movement of infective vectors on to the new crop has been observed where groundnuts have been planted after groundnuts. Rosetted dry-season self-set plants adjacent to infested and rosetted early-rainy-season self-set plants occurred between rows of new crop, which soon became infested and rosetted.

Thus self-set groundnuts can be a most important source of virus and vector for the carry-over of the disease from one season to another. In the 1951–2 season, groundnuts following groundnuts were often severely attacked by rosette disease early in the season, and several of these fields were sources of winged aphids which later infested adjacent fields that had not been down to groundnuts the previous year.

THE BIOLOGY OF THE VECTOR

Little is known of the range of wild host plants of *A. craccivora* or what part they play in the biology of the pest. Neither is it known if any can harbour the virus of rosette disease. Soyer (1939) records three species in the Belgian Congo, *Indigofera hirsuta*, *Euphorbia hirta*, and *Centrosema plumeri*. All three were tested to see if they would take the virus, but only *C. plumeri* showed mosaic symptoms, and attempts to transmit the virus back to groundnuts failed.

During the rainy season and dry season of 1950 in the Southern Province, wild plants were observed with colonies of *Aphis craccivora*. Their occurrence may sometimes have been due to temporary local colonization on regenerating plants. Most species of the alternate hosts belong to the Papilionaceae, which family includes the groundnut.

List of alternate hosts

PAPILIONACEAE: *Vigna reticulata* Hook, *V. triloba* Walp., *V. unguiculata*, *Crotalaria fwamboensis* Bak., *C. intermedia* Kotschy, *C. kirkii* Bak., *Dolichos malasanus* Bak., *D. fulcatus*, *Indigofera rhynchocarpa* Welw. ex Bak., *Dalbergia melanoxylon* Guill. & Perr., *Cajanus cajanus*, *Rynchosia resinosa* (Hockst. ex Rich) Baker, *Millettia makondensis* Harms.,

CAESALPINIACEAE: *Cassia absus* L., *Brachystegia burtii* Hoyle.

MIMOSACEAE: *Dichrostachys nyassana* Taub.

PORTULACACEAE: *Talinum caffrum* (Thunb) Eck. & Zey.

LOGANACEAE: *Strychnos subifera* Gilg. & Busse.

(The *Vigna* and *Crotalaria* spp. and *Cassia absus* are annuals: the rest are perennials.)

Aphis craccivora thrives on all three species of *Vigna*. *V. reticulata* and *V. triloba* grow actively even during the dry season in moist valley bottoms, and can support colonies of *Aphis craccivora*. Successful cross-transferences from both species to groundnuts and vice versa have been carried out. A planting of cowpea, var. Saunders Upright, was very heavily infested during March 1950.

Dolichos malasanus was first found infested in late February 1950. The few clumps that carried colonies were composed of new growth which had sprung up after land was prepared for maize and groundnuts. This species died down during the dry season but grew again during the November rains. Heavy infestations were found on the inflorescence, which begins to open before the leaves are fully expanded, and on the young leaves. Cross-transferences have been made.

The three *Crotalaria* spp. were found infested only occasionally during the late part of the rainy season when in flower and rarely during the dry season. *Crotalaria* plants were found during the dry season only on land that had been ploughed after harvest, the seeds germinating in the deeper moist soil into which they had been turned. *Talinum caffrum* also was found infested only during the later part of the rainy season. This species survives the dry season as a fleshy tuber and quickly puts out new growth with the first rains, but none of the numerous specimens examined during November was infested. *Rynchesia resinosa* and *Cassia absus* were also found infested during the later part of the rainy season but successful colonies were never found on either of these species. Both have many hairs which secrete a viscous globule at their tips. Plants were often observed bearing many alatae caught in this material, but only after periods of heavy rain were first-instar nymphs observed. It appeared that this temporary breeding was possible because the rain had washed off the viscous material, which killed the aphids when secretion was renewed.

Regenerated plants of *Millettia* or *Lonchocarpus* sp. were found carrying colonies from February through the dry season and during the rains. On *Millettia makondensis* only occasionally were adults with a few young nymphs found, never a successful colony.

Flourishing colonies were found on regenerated *Dichrostachys nyassana*, *Strychnos subifera* and *Brachystegia burtii* only at the end of the rainy season. Regenerated *Dahlbergia melanoxylon* carried colonies at the end of the rainy season and again early in the rains.

Only a few specimens of the many *Indigofera rhynchocarpa* studied carried flourishing colonies and then only on inflorescences. This species normally flowers in November, but infested specimens were found flowering in February on cultivated land, i.e. on regrowth after stems were destroyed by cultivation.

Most of the observations were made on land under cultivation or cleared land that had been abandoned because it could not be conserved; they show how the aphid survives through the dry season on the disturbed flora, when young tender growth favoured survival more than the natural condition of the bush. Several of the species recorded as alternate hosts were probably sites of accidental temporary infestations, e.g. *Strychnos subifera*, which is decidous, *Dichrostachys nyassana* and *Dalbergia melanoxylon*, which carry mature foliage in the dry season.

The important alternate hosts which carry the aphid through the dry season are *Vigna* spp. and *Millettia* or *Lonchocarpus* sp., while *Vigna* spp. *Dolichos malasanus* and perhaps *Indigofera rhynchocarpa* are important early in the rains. *Brachystegia burtii* and perhaps other species of *Brachystegia* may be important dry-season hosts because they produce a succession of young leaves.

The reduction in aphid populations caused by predators has already been mentioned. Colonies of about 100 aphids placed on uninfested plants were sometimes found to be rapidly exterminated by predators, chiefly Syrphidae. To test whether colonies initiated by single adults could be easily discovered by predators twelve colonies consisting of one adult and five nymphs were evenly spaced over an acre. Eight of the colonies were established on the next day. Predators, either adult coccinellids or eggs of syrphids or coccinellids, were present on two colonies by the second day, on a further five by the third and on the eighth by the fourth day. Thus even very small colonies can be rapidly found by predators.

The aphids increased in number for the first few days, as did the predators. On six of the colonies the predators were sufficiently numerous to check the multiplication of the aphids by the 5th–6th day and exterminate the colonies by the 9th–10th day (Fig. 2). On the remaining two colonies the aphids established themselves more successfully and, in spite of reductions in numbers after 9–12 days, increased again; but further additions to the predator populations caused extinction after 23 and 24 days.

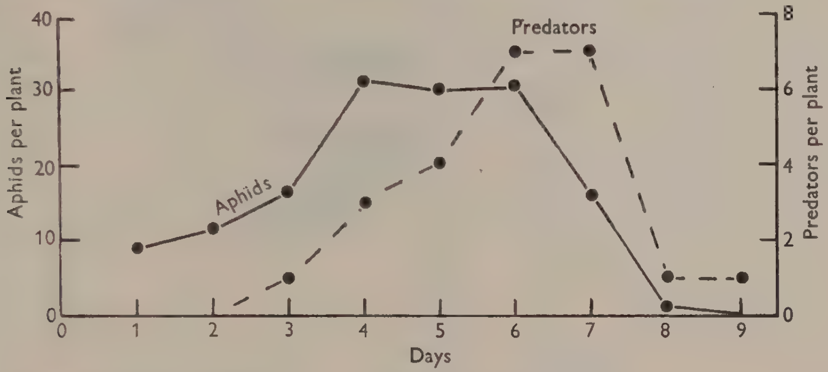

Fig. 2. The reduction of an initially small aphid colony by predators.

The greater increase in the number of rosetted uninfested plants compared with that of rosetted but infested plants (Table 1) was probably caused by the destruction of numerous small colonies by predators.

VARIETAL RESISTANCE

Twenty-five varieties of groundnuts were planted in 1951 and became infected with rosette virus. Frequent counts were made to see if any varieties showed resistance. A final count 53 days after planting showed a wide range of infection over the different varieties (Table 4). Part of the wide variation between plots of the same variety was due to secondary spread of apterous adults from neighbouring plots. Rejecting the two outer rows of each plot from the counts only partially eliminated this source of error. Of the varieties with the lowest incidence of infection, three, Mwitunde Urambo, Mwitunde Ukiriguru and Mwanza Y came from north-west Tanganyika. In a similar trial at Urambo in 1949 two varieties of the Mwitunde group, Mwitunde Nyaluki and Mwitunde Nyasaland, came 15th and 23rd in order of infection. The low incidence of infection in the Mwitunde group, which is cultivated by native growers in north-west Tanganyika, suggests that there had been some natural selection.

Further, the spatial distribution of the disease at the junction of Mwitunde plots with other, heavily infected, varieties, indicated some form of resistance (see Fig. 3).

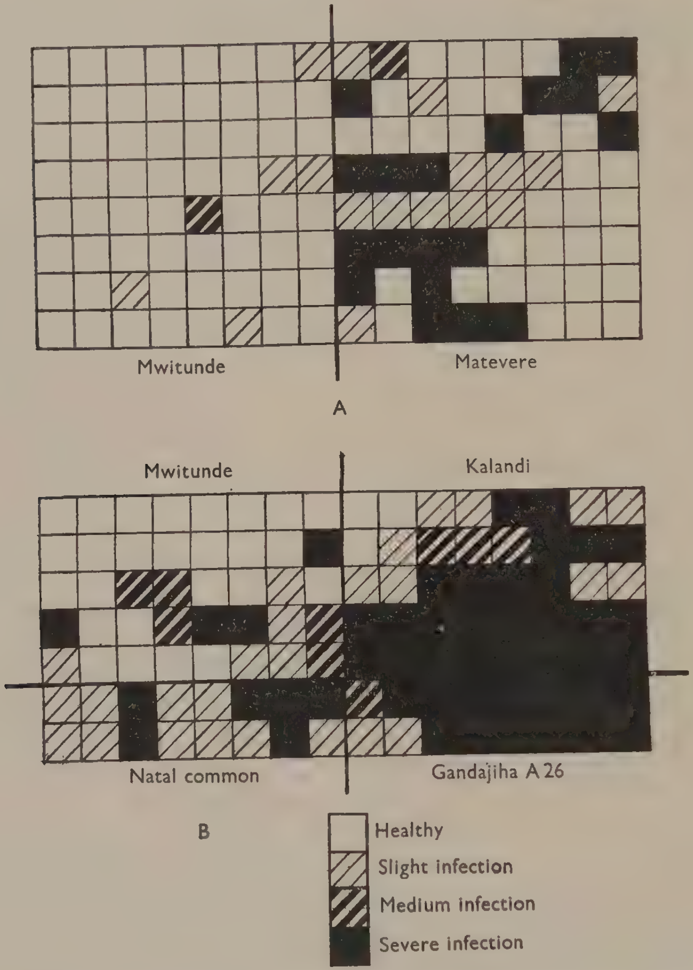
Mwitunde
Matevere
A
Mwitunde
Kalandi
Natal common
Gandajiha A 26
B
Healthy
Slight infection
Medium infection
Severe infection

Fig. 3. The apparent 'resistance' of the variety Mwitunde to rosette disease at the borders of adjacent susceptible varieties.

In another variety trial in 1952 (Table 4) the two Mwitunde varieties were not less infected than the other varieties for the first two counts, i.e. during the phase of primary infection by winged aphids coming from outside the planted area. A χ^2 analysis of these results shows that the initial aphid infestation was no lower on the Mwitunde plots than on the others. By 14 February secondary spread had occurred and some varieties had many infested plants.

TABLE 4. *The incidence of rosette disease on varieties of groundnuts*

(Plot size: 8 rows × 50 links.)

	Infected plants per six plots					Plots with secondary spread			
	5. iii. 1951	26. i. 1952	1. ii. 1952	8. ii. 1952	14. ii. 1952	14. ii. 1952	21. ii. 1952	28. ii. 1952	6. iii. 1952
Natal Common	296	10	14	50	255	6	6	6	6
Kano	148	6	10	17	70	1	2	3	5
Cabri	120	7	10	22	134	3	3	3	4
Kano 38	116	9	14	21	114	4	5	6	6
Samaru 38	644	14	16	26	113	1	6	6	6
Hongkong	168	6	9	26	161	4	4	6	6
Mbambey 29–56	—	8	14	37	166	5	5	5	6
Mbambey 29–70	—	8	16	96	241	4	5	6	6
Amani A5	—	6	10	13	90	2	6	6	6
Amani A22	—	11	14	21	84	2	3	3	6
Amani A25	—	10	15	27	124	3	3	5	6
Ashford Sudan	232	11	18	49	127	2	3	4	5
American Sudan 1	136	12	17	64	248	2	2	4	5
Baladi Bunch	732	11	16	31	136	3	5	5	6
Giza Bunch	—	12	12	23	89	3	4	4	5
43b-90a-Ny-J10	—	7	13	19	107	2	2	3	5
Mgongo	—	9	13	50	113	3	5	5	5
Miloya	528	8	9	19	108	2	3	5	6
Virginia Bunch	—	5	8	16	74	2	3	5	6
Kanyoma	248	16	23	98	261	4	6	6	6
Tunduru A.	—	14	21	58	247	4	5	5	6
Wima Bunch	432	12	16	29	119	3	4	4	6
Matevere	208	10	16	26	87	1	1	2	5
Mwitunde Urambo	116	7	14	25	74	0	0	0	1
Mwitunde Ukiriguru	64	6	15	20	69	0	0	0	0

TABLE 5. *Distribution of aphid infestation on sixty plants of six varieties, 27 January* 1952

	Uninfested	One adult present	Small colony present	Vigorous colony present
Natal Common	44	3	13	0
Ashford Sudan	41	3	15	1
Tunduru A	32	6	19	3
Virginia Bunch	48	4	8	0
Mwitunde Urambo	30	6	24	0
Mwitunde Ukiriguru	44	4	12	0

Plants of one variety were sometimes infected by apterous adults that walked from a neighbouring variety; and also by occasional winged forms.

The major loss produced by rosette occurs as a result of this secondary spread. The patches can be large or small but for simplicity, a plot was recorded as having secondary spread present or absent. By 6 March five or all six of the plots of twenty-two varieties had secondary spread, one had four plots so affected, while Mwitunde Urambo had only one and Mwitunde Ukiriguru none.

The differences shown in the infection data are reflected in the yields (Table 6). The two Mwitunde varieties yielded well, both higher, but not significantly so, than the short-season Natal Common, which is the best variety so far known for the area. The Mwitundes are long-season varieties; Cabri and Kano both short-season varieties yielded fairly well but less than the Mwitundes. The other varieties were all long-season and their yields were relatively low.

The orthodox small-plot experiment is not suitable for testing varietal resistance to rosette, as the primary infections are not usually normally distributed. Also, differences between varieties are lessened by aphids moving from heavily to lightly infested plots. To obviate these difficulties the following type of experiment was made. A field was planted to Natal Common at 34 in. between rows and 4½ in. between seeds. Immediately after, the varieties to be tested were planted as follows: five seeds of the variety under test were substituted for Natal Common seeds at a spacing of 6 ft. at sites marked with stakes. These five seeds spread over a 30 ft. row of Natal Common planting constituted a plot. There were five treatments: Mwitunde Urambo and Mwitunde Ukiriguru from individual healthy plants selected from the variety of trial of 1951; the seed of the same two varieties taken from stock; and Natal Common as a standard. The blocks of plots were separated from each other by six rows of Natal Common. The plants under test were infested with two apterous adults taken from naturally infested diseased plants. In this way, a uniform initial infection could be produced and secondary spread could be estimated by the number of adjacent plants of a susceptible variety becoming infested.

Two such experiments were made. On one, plants were infested 9 days, and on the other 21 days, after emergence. The early-infection experiment was examined for aphids 10 and 15 days after infestation. The infestations varied from one small nymph to a flourishing colony, with and without larval or adult predators. To simplify recording, plants were recorded as infested or not infested (Table 7).

Natal Common had significantly fewer uninfested plants per plot than the Mwitundes. The plants were too large on the late-infection experiment for uninfested plants to be assessed accurately.

The number of infested plants which became diseased were recorded (Table 8). In both experiments, plants of Mwitunde Urambo grown from stock seed gave the fewest rosetted plants per plot. In the early-infection experiment it was highly significantly less than Natal Common, but not significantly less than the other Mwitunde type. In the late-infection experiment it was highly significantly less than Natal Common and the other Mwitunde type. Further work is necessary to confirm the above results.

The amount of secondary spread was recorded on both experiments 45 days after infestation.

In both experiments significantly more secondary spread occurred in Natal Common than in the Mwitunde types.

Storey & Bottomley (1928) described two expressions of rosette disease. In so-

TABLE 6. *Yields of certain groundnut varieties (kernels, lb./acre)*

Variety	Yield
Mwitunde Ukiriguru	955
Mwitunde Urambo	934
Natal Common	864
Cabri	825
Kano	700
Others as Table 4	349–631
Sig. diff., 5 %	116
Sig. diff., 1 %	221

TABLE 7. *Number of plants per plot not infested at both counts*

(Data transformed for analysis as $\sqrt{(x+0{\cdot}5)}$.)

	Natal Common. Stock	Mwitunde			
		Ukiriguru		Urambo	
		Selected seed	Stock seed	Selected seed	Stock seed
Actual means	0·7	2·2	1·5	1·6	1·6
Transformation	1·05	1·60	1·37	1·34	1·41

Significant differences (transformed values): 5 %, 0·18; 1 %, 0·24.

TABLE 8. *Number of rosetted plants per plot*

	Natal Common. Stock	Mwitunde			
		Ukiriguru		Urambo	
		Selected seed	Stock seed	Selected seed	Stock seed
Early infection	4·1	3·6	3·4	3·3	2·9
Late infection	3·7	3·9	3·7	4·0	2·6

Significant differences	Early	Late
5 %	0·43	0·30
1 %	0·58	0·41

TABLE 9. *Number of Natal Common plants per plot infected with rosette after 45 days*

	Natal Common. Stock	Mwitunde			
		Ukiriguru		Urambo	
		Selected seed	Stock seed	Selected seed	Stock seed
Early infection	14·4	3·8	2·2	2·3	1·6
Late infection	9·3	3·7	3·5	5·0	3·3

Significant difference at 1 %: early, 2·3; late 3·3.

called 'normal rosette' the first affected leaf shows a faint indefinite mottle, the next leaf to open is predominantly pale yellow and the veins form a green network; later-formed leaves are progressively smaller, chlorotic (often uniformly yellow without dark veins) curled and distorted; the axis ceases to elongate after chlorotic symptoms first appear. With the 'mosaic type', the chlorosis is not complete but is interrupted by areas of normal green tissue, and there is less stunting.

The symptoms on the variety collection (ninety-eight varieties) on the experimental farm are predominantly those described above as normal rosette, but the mosaic type occurs. There are two exceptions, Mwitunde Urambo and Mwitunde Ukiriguru, both of which show quite distinct symptoms from those of the other varieties, from Brazil, U.S.A., Dutch East Indies, Sudan, Senegal, Congo and other regions of Africa.

The first affected leaf shows a few pale brownish green areas with a brown centre. The next leaf to open is slightly reduced in size, of an uneven pale brownish green with brown spots. Instead of being turgid, as for normal rosette, it is flaccid and the leaflets fall off if not handled carefully. When this condition was first noticed it was thought that a new virus was concerned. The next leaf to unfold is still more reduced in size and shows similar symptoms. Later leaves, however, are quite turgid and when just expanded are pale green with paler interveinal clearing. As they age, some of the paler interveinal areas become chlorotic and the rest of the leaf dark green. The degree of internodal shortening varies considerably from plant to plant even when infected at the same age. The symptoms show much more quickly on the Mwitunde varieties than on Natal Common; 50% of the Mwitundes showed symptoms 9 days after infection compared with 13 days for Natal Common.

The above symptoms are produced by infection with aphids taken from typical Natal Common infections, and aphids reared on infected Mwitunde and allowed to feed on Natal Common will produce the typical symptoms of this variety.

CHEMICAL CONTROL

Preliminary trials with systemic insecticides were made during 1949 at Kongwa.

The first experiment, using schradan, compared application to the soil with application to the leaf surface at rates of 0·2 and 0·4 ml. of 0·2% (v/v) commercial concentrate per plant. Five randomized blocks of these treatments and controls were set up. Two days after treatment, four apterous aphids were placed on each plant. Counts were then made daily.

No significant differences were noted between the controls and the two soil treatments, and observations on these ceased after 6 days when the plants carried populations of 70–90 aphids. At this time the plants receiving 0·2 and 0·4 ml. of insecticide on the foliage (treatments P 1 and P 2) had averages of 3 and 0 aphids respectively. Three of the P 1 plants were free from aphids. The next day all five P 2 plants and the three clean P 1 plants were re-infested with four apterous aphids. Six days later all were free from aphids. They were re-infested 2 days later but this time the aphids became established. Four days later the observations ended, as the plants were outgrowing their pots. Even at this time, 21 days after application, the insecticide was probably having some effect as treated plants had only 183 aphids 4 days after the final re-infestation, whereas the controls had 393 on the 4th day of the first infestation.

The second experiment tested six commercial phosphonous acid derivatives and a commercial preparation of hexaethyl tetraphosphate. The phosphonous acid preparations were applied at 0·5 ml. per plant of a 1% (v/v) solution in 0·1% wetter, and the hexaethyl tetraphosphate at 0·5 ml. per plant, using the manufacturer's recommended dilution. The solutions were painted on the plants with a palette brush to ensure that the complete dose was applied. Four apterous aphids were placed on each plant at the beginning of the experiment and again on the 4th, 9th, 14th, 19th and 24th day after treatment (Table 10).

TABLE 10. *Total aphids on four plants at varying periods after the application of insecticides*

Days after treatment	Phosphonous acid derivatives						Hexaethyl tetraphosphate	Control
	A	B	C	D	E	F		
3	0	0	0	2	0	0	1	87
9	5	0	0	0	0	0	87	190
14	2	0	0	4	2	3	—	—
19	10	2	0	43	14	33	—	—
24	100	2	5	141	35	58	—	—

All treatments were aphicidal for 3 days, but then haxaethyl tetraphosphate became non-effective and aphids increased on this treatment and on the control so rapidly that counting was discontinued after the 10th day. Twenty-four days after treatment the infestations were still small on B and C. Treatments E and F were still exerting an effect, but A and D were almost ineffective.

Unfortunately a high wind blew a number of cages over and tore the muslin covers so that the development of infestation can only be described in general terms after the 24th day. On the 29th day there were too many aphids to count on treatment D and on two plants of F. On A, B, C and E there were averages of 76, 12, 16 and 10 aphids. The experiment was discontinued after 40 days; by this time the infestations had become extremely variable within as well as between the treatments. The final state of infestation was:

A: One plant with 68 aphids, two plants uncountable.
B: infestations of 0, 8, 59 and 79.
C: infestations of 1 and 67 with one plant uncountable.
E: infestations of 1 and 68 with one plant uncountable.

During the 1951 season two field trials were made in the Southern Province with a commercial preparation based on schradan. A spray containing 0·5% active ingredient in 0·1% wetter at a rate of 40 gal./acre was used in a randomized block design of the following:

Control
Sprayed 23 days after planting.
Sprayed 41 days after planting.
Sprayed 23 and 41 days after planting.

Plot size was eight rows, 28 in. wide by 70 links long, with eight replicates. Twelve days after the first spraying infected plants were counted and recorded as infected before spraying. This count was subtracted from subsequent counts which were made at 6-day intervals. Fig. 4 shows the results obtained per plot of six rows, edge rows being discarded.

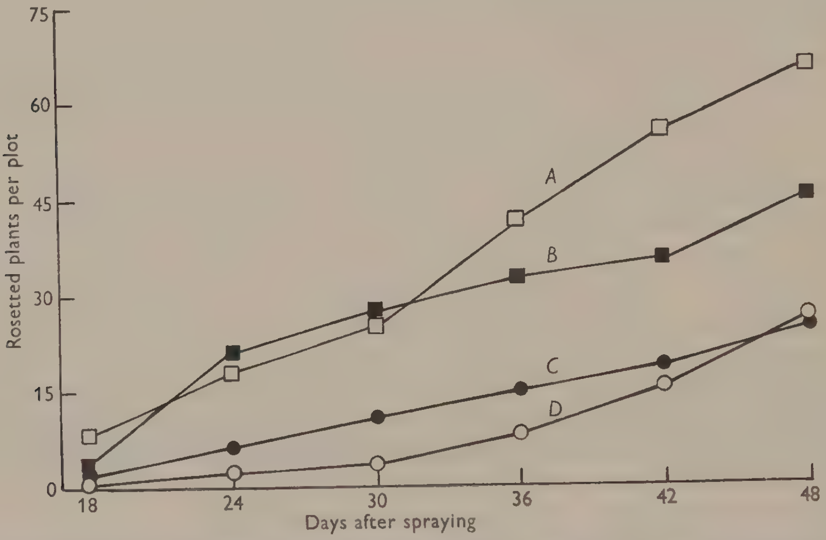

Fig. 4. The control of rosette disease by schradan. *A*, control; *B*, one spraying 41 days after planting; *C*, two sprayings 23 and 41 days after planting; *D*, one spraying 23 days after planting.

Up to the 18th day after the first spraying there are only two comparisons, unsprayed and first spraying only. The early sprayings reduced the incidence of rosette disease considerably. The higher incidence at this time on the plots to be sprayed twice is due to a high incidence on one plot. This plot contributed from one-third to one-half of the rosetted plants for the treatment total as a result of spread of aphids from the adjacent unsprayed plot. The later spraying by itself checked the increase in number of rosetted plants compared with the controls but following the early spraying it had no appreciable effect. By the 48th day the rate of increase was uniform on all treatments, as a result of vectors moving from untreated plots and the surrounding stand, and counting was discontinued. Also the insecticide in the plant had been considerably diluted by plant growth and some had probably decomposed.

The insecticide probably exerted its effect by killing infective aphids already on the plant, and also alatae which flew in from surrounding areas. The incoming aphids might infect the plant on which they alighted but would cause no further infections. This flight of winged aphids from surrounding infected areas means that the value of the insecticide cannot be judged from final infection figures at harvest, or from yield results, but only on the comparative figures obtained during a short period over which the experimental design is valid. Judged in this way insecticides based on schradan promise well for rosette control.

Rosette disease shows a patchy distribution which produces high standard errors and may obscure useful results. An attempt was made to overcome this difficulty in an experiment comparing three concentrations of schradan, 0·1, 0·3 and 0·5%. An untreated 50-link row formed a buffer between each treatment and control plot, also, along the rows of the experimental area, the experimental plots were staggered from block to block to give a chequer-board pattern of non-experimental interstitial rows as a check on the natural distribution of the disease. Sixty-four blocks were laid out in an 8 × 8 square, each block containing four experimental rows and four interstitial rows.

The rosette on the control plots was compared with the mean of that on the adjacent interstitial plots after 11 days and the results showed that there is no relationship between them. Thus the initial distribution of rosette on this area was not particularly patchy. The frequency distribution of the number of rosetted plants per row for the interstitial area could not be fitted to either a binomial or a Poisson distribution. The results obtained were therefore not submitted to an analysis of variance and are presented in Table 11.

TABLE 11. *Frequency distributions of the number of rosetted plants per plot 25 days after spraying, for three concentrations of schradan*

	No. of rosetted plants per row						
Concentration	0	1	2	3	4	+4	Mean per plot
0·5	51	8	4	0	1	0	0·31
0·3	36	18	6	4	0	0	0·66
0·1	40	16	4	2	0	2	0·73
Control	34	18	6	2	1	3	0·97
Interstitial rows	31	20	7	4	1	1	0·90

Only the highest concentration of insecticide reduced the incidence of rosette. It was noticed that generally on the farm there was but little increase in rosette disease at this time, so that the incidence was much lower than expected, being only about 0·6% on the untreated plots. It was caused by a few alatae flying in from distant infected sources. Individual plants sprayed with the three concentrations of schradan were infested with about 100 aphids to determine speed of kill. Aphids and predators were counted until the 6th day (Fig. 5). For the 0·5% concentration all the aphids were dead within 24 hr., for the 0·3 and 0·1% concentrations all were dead 2 and 3 days after spraying. On the control plants, predators killed the aphids by the 6th day. Another and heavier infestation was made on the 7th day after spraying. Concentrations of 0·5 and 0·3% eliminated the aphids by the 4th and 5th day after infestation. The 0·1% concentration caused some reduction by the 4th day; but numbers then remained constant until the 8th day and then rapidly declined until all were gone on the 13th. Predators, which were numerous by the 8th day, were responsible. Further observations on the degree of toxicity of the plants could not be made as the plants were so badly rosetted that even approximate counts could not be made without disturbing the colonies and causing aphids to fall from the plants.

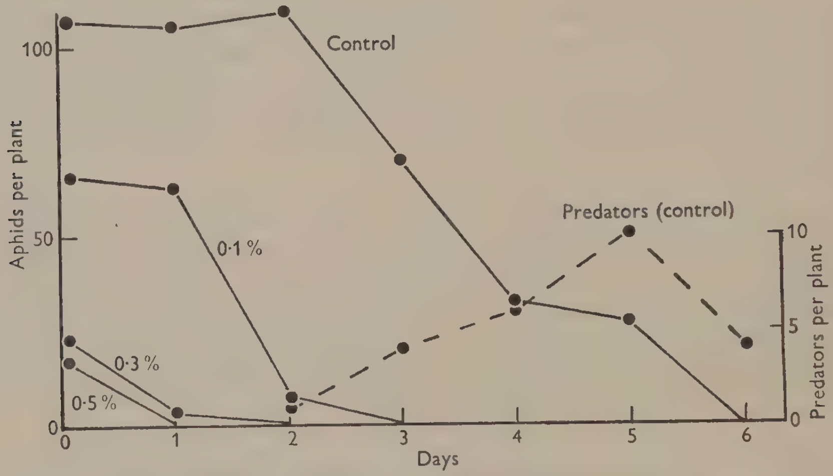

Fig. 5. The reduction of aphid colonies due to schradan and predators.

Discussion

In the Southern Province of Tanganyika there is a range of indigenous host plants on which the groundnut aphid lives, and locally high populations can build up early in the rainy season on the young shoots of the perennial *Dolichos malasanus* which is widely distributed. *Indigofera rhynchocarpa*, which has been found in several habitats, can also support colonies of aphids early in the rainy season. Although sources of the vector of rosette disease have been found, no reservoir of the virus has been found in the native vegetation. That such a reservoir exists is not certain; the early infections noted from 1948 onwards may have come from plantings of groundnuts that existed in the area before the Corporation began its operations, and remained in dry-season volunteer plants.

The control of rosette appears to centre on eliminating dry-season self-set plants and on controlling aphids which come from the surrounding natural vegetation early in the season.

Much can be done to control the self-set plants by cultivations before the new crop is planted. Some soil types can be ploughed before the rains begin; others, however, cannot be ploughed until the top 9–12 in. of soil has been moistened. The aim here must be to turn in the vegetation before vector and virus have passed to the new flush of self-set plants. Small pockets of infected plants may remain and the most satisfactory method of eliminating self-set plants would be to plant only varieties that have a seed-dormancy period. Only long-season varieties possess this valuable character, and unfortunately they do not yield as well as Natal Common. Work is now in progress to try and produce high-yielding strains of such varieties.

The control of the aphid is less straight-forward. Predators are numerous and varied, but insufficient to be relied upon unaided. Their efficiency might, however, be enhanced. First, they might be deliberately bred and released early in the season. Secondly, plants carrying species of aphids of no economic importance might be grown, to build up the general level of the local predator population. Thirdly, strains of groundnuts which are not so suitable as hosts for the aphids might be bred, thus increasing the relative proportions of predators to aphids. Fourthly, enough of a systemic insecticide might be introduced into the groundnut seed to give some protection during the first 2 or 3 weeks of growth.

The first method is practicable but expensive, and its value would depend upon the crop carrying a high cash return per acre. The second method would be much cheaper and might be feasible. The aphid fauna of the region is poor and bush surveys have shown that species are few and rare. There is, however, one exception: large patches of *Vernonia glabra* (Steetz) Vatke carried many large black aphids from February to June. The plants seeded in June and the aphids disappeared as the stems dried. New basal shoots appeared in August before the beginning of the rains. The large black aphid, however, did not reappear immediately; some small colonies of another species appeared but did not produce a general infestation. A population of predators on another host during February would be of no value for controlling the groundnut aphid. Occasionally, however, during the latter part of the dry season, green *V. glabra* plants in moist valleys carried populations of both species of aphid and of attendant predators. By encouraging *V. glabra* in such situations high populations of aphids and predators might be built up in October and November, and a proportion of the predators might transfer their attentions to the groundnut aphids on *Dolichos malasanum* and other hosts in the bush and on the early groundnut plantings. Many attempts were made to transfer the groundnut aphid to *Vernonia glabra* and the two *Vernonia* aphids to groundnuts but all failed.

Plants of the Mwitunde type carried an early aphid infestation of the same order as four other varieties. The final incidence of rosette, however, was higher on the latter varieties, both for early counts of individual rosetted plants and for the amount of secondary spread. The Mwitunde types seem to have a character that mitigates the severity of the disease. This character almost certainly functions indirectly; when secondary spread starts from the edge row of a susceptible variety on to a plot of Mwitunde, this variety can become heavily infected over a limited area. Infestation of individual plants with infected aphids gives the same proportion of rosetted plants as does Natal Common. The variety may resist infection but this seems unlikely. It is more likely that the Mwitunde types are less favourable hosts for the aphids than the other varieties. Thus the colonies on Mwitunde may increase less quickly, so that predators can exterminate them before they are large enough to disperse and produce secondary spread. The Mwitunde types grown in the Corporation's areas are clearly mixed and selection might produce high-yielding strains resistant to the aphid.

Tsi (1950) has reported that certain seeds absorb schradan and the resulting seedlings are toxic to aphids for a few weeks after germination. Groundnut seeds have been soaked in this systemic insecticide and the resulting seedlings were toxic to aphids for 21 days when plants were grown in the shade. The treated seeds germinated poorly; this may have been due to the insecticide itself or to the adjuvants which amounted to 60–65 %. A purer material is now available for testing in the field. This line of control is not so hazardous or expensive as field spraying.

I am very grateful to Mr P. Greenway of the East African Herbarium, who identified the alternate hosts for me.

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STUDIES IN *RUBUS* VIRUS DISEASES

VI. APHID TRANSMISSION OF RASPBERRY LEAF MOTTLE VIRUS

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Raspberry leaf mottle virus is acquired by *Amphorophora rubi* in feeding periods of ½–2 hr. Longer feeding periods cause no significant fluctuations in infectivity. During continuous feeding on healthy plants, infectivity declines rapidly after 2 hr., but persists for at least 5 hr. if aphids are transferred frequently to fresh healthy plants. The estimated probability of infection by single aphids is 1 in 20. Rarely more than 50% of test plants, *Rubus occidentalis*, seedlings became infected, and this is thought to be due mainly to variation in plant susceptibility. Young leaves of infected Norfolk Giant are, however, better sources of virus than older leaves.

Two aphid species commonly infest raspberries in Scotland, *Amphorophora rubi* Kalt. and *Aphis idaei* v.d.G. The first of these transmits raspberry leaf mottle, vein-banding and leaf spot viruses (Cadman, 1951, 1952*b*, *c*), whereas *A. idaei* is only known to be a vector of moderate vein chlorosis (Cadman & Hill, 1947; Cadman, 1952*a*). *Amphorophora rubi* was shown to acquire leaf-mottle virus in feeding periods of less than 24 hr. and to retain infectivity for only a few hours. Little was discovered of the vector relations of vein-banding, leaf spot and moderate vein chlorosis except that the acquisition periods for all three were less than 24 hr.

The work reported here was begun with the objects of clarifying these relations; of ascertaining the vector relations of other known raspberry viruses; and of discovering whether aphids other than *A. rubi* and *Aphis idaei* could act as vectors. Leaf-mottle virus was chosen for detailed study, partly for convenience and partly because exploratory work had suggested that the relations of this virus with *Amphorophora rubi* were essentially similar to those of other viruses transmitted by this aphid.

MATERIAL AND METHODS

Rubus idaeus varieties, and seedlings from these, are not readily infected by means of aphids, and are therefore unsuitable test plants for analytical work requiring high rates of transmission by small numbers of aphids. *R. occidentalis*, the North American black raspberry, on the other hand, is more susceptible to infection (Cadman, 1951), and consistent results are obtainable with this host using groups of ten aphids or fewer per plant. In all the experiments described below very young seedlings of *R. occidentalis* var. Cumberland were used as test plants. For preference, seedlings that had expanded one true leaf were used because these were more readily infected than older plants. During the summer such plants developed symptoms of leaf mottle 8–9 days after infection. These consisted in down-curling of the leaves and petioles, distortion of the laminae and appearance of diffuse chlorotic blotches. The plants afterwards recovered somewhat and showed chronic symptoms similar to those described and illustrated by Cadman (1951).

Difficulties were encountered in maintaining a constant supply of seedlings throughout the season. Seeds of *R. occidentalis*, like those of other *Rubus* spp., will not germinate unless first subjected to a lengthy period of alternating high and low temperatures. This is usually provided for by stratifying the seeds in moist sand or peat directly after harvesting and storing them either in a cold frame or outdoors over winter. Seeds so treated begin to germinate in the following spring and will sometimes continue germinating until midsummer. A more reliable technique is very desirable but the problem of dormancy in *Rubus* seeds has received little attention.

Virus free colonies of *Amphorophora rubi* and *Aphis idaei* were reared on *Rubus idaeus* seedlings. Colonies of *Aphis ruborum* Börner and *Amphorophora rubi* Börner, kindly supplied by Dr H. J. de Fluiter, Wageningen, were reared on plants of *Rubus caesius* collected in the field. Stocks of *Macrosiphum euphorbiae* were maintained on seedling *Fragaria vesca* but *Macrosiphum fragariae* were collected from a garden plant of *Rubus procerus* var. Himalaya Giant. All the experiments were carried out in an unheated and unprotected glasshouse in which aphis infestation was controlled by routine nicotine fumigations. Appropriate controls were included in each set of experiments, but none of these became infected. Test plants were fumigated at the conclusion of each stage of an experiment.

Throughout, detached leaves or whole plants of Norfolk Giant (East Malling L234 clone) were used as sources of leaf mottle virus.

RESULTS

Cadman (1951) reported transmission of leaf mottle by *Amphorophora rubi* to *Rubus saxatilis* and *R. occidentalis* and thought that *Aphis idaei* was not a vector. In experiments made in 1951 and 1952 no transmissions were obtained with *A. idaei*, *Amphorophora ruborum*, *Macrosiphum fragariae* or *Amphorophora rubi* Börner under conditions where *A. rubi* Kalt. infected 50% of the test plants. It is therefore concluded that none of these aphids is a vector of leaf mottle. In a single trial, *Macrosiphum euphorbiae* from an outdoor colony on Norfolk Giant infected one of ten test plants, but this result was not confirmed by three subsequent experiments. The failure of the bramble strain of *Amphorophora* (*A. rubi* Börner) to transmit suggests an intrinsic difference between this and the raspberry strain (*A. idaei* Börner), although neither is accorded specific rank by Lambers (1950). There is little doubt that *A. rubi* is the principal and perhaps only vector of leaf mottle, and detailed experiments were made only with this aphid.

The experiments were modelled on those of Watson (1940) with a sugar-beet yellows virus and *Myzus persicae*, and were designed to test the infectivity of aphids after varying times of feeding on infected and healthy plants. They divide conveniently into two groups. In the first, each aphid group was tested for its performance on a single healthy plant; and in the second, each group was tested on a succession of two or more healthy plants.

Infectivity of aphids tested on single healthy plants

An experiment to test the interactions between feeding times on infected and healthy plants was arranged on a factorial design. Detached leaves of Norfolk Giant were the source of infection and the treatments and results are shown in Table 1. Increasing the infection feeding period from ½ to 2 hr. caused a sharp rise in infectivity of the vector, but infection feeding periods longer than this caused no significant fluctuations in infectivity. There was no indication of any delay period during which aphids were unable to transmit virus. Aphids infected as many plants after 1 hr. as after 24 hr. test feeding.

TABLE 1. *Number of plants infected by groups of ten aphids after varying feeding times on infected and healthy plants*

(Ten plants per treatment repeated on five occasions.)

T.F.P.* (hr.)	I.F.P.* (hr.)						Mean percentage infection
	½	2	6	10	24	Total	
1	2	25	25	12	23	87	35
4	2	16	17	21	19	75	30
24	2	21	25	27	16	91	36
Total	6	62	67	60	58	253	—
Mean percentage infection	4	41	45	40	39	—	—

* I.F.P. = infection feeding period. T.F.P. = test feeding period on healthy plants.

Attempts to investigate the effects of starvation before infection feeding and of short infection feeds gave inconsistent results. No infections were obtained in either of two trials designed to compare the performance of starved and unstarved aphids following infection feeds of 5 min., 30 min., 1 hr. and 2 hr. duration. On the other hand, infections were obtained once following a 5 min. and once following a 30 min. infection feed in an experiment testing all combinations of 5, 15 and 30 min. infection and test feeding periods.

A puzzling feature of Table 1 is that on only one occasion did more than 50% of the healthy plants become infected. The results of two other experiments offer some explanation for this. The first was designed to determine the probability of infection by a single aphid. Aphids fed on detached Norfolk Giant leaves for 24 hr. were placed in groups of 1, 5, 10 and 20 per plant on each of four lots of 10 *Rubus occidentalis* seedlings. No special precautions were taken to randomize the treatments among the plants, but the whole experiment was repeated on six occasions between June and August 1951. The results (Table 2) show that approximately

1 in 7 aphids in the single aphid group transmitted virus. The data from all groups provide a maximum likelihood estimate of the probability of infection by a single aphid, $p = 0{\cdot}0496 \pm 0{\cdot}0046$, i.e. on the average 1 in 20 aphids transmitted virus. The method of estimation assumes (1) that infections caused by larger groups of aphids are local and independent of the number of aphids in the group, and (2) a constant level of susceptibility of the healthy plants (Watson, 1936). Agreement between the expected numbers of infections and those observed is poor, particularly in the cases of the 1 and 20 aphid groups ($\chi^2 = 33{\cdot}48$, $P < 0{\cdot}001$). If values for p and the corresponding numbers of infections are calculated for each replicate of the experiment there is a slightly better fit between the observed and calculated totals ($\chi^2 = 22{\cdot}55$, $P < 0{\cdot}001$). Values of p vary between 0·01 and 0·17, and this is held to imply variation in plant susceptibility as a major cause of discrepancy.

TABLE 2. *Numbers of infections caused by aphid groups of varying numbers*

(Ten plants per treatment repeated on six occasions.)

	No. of aphids per plant				Probability of infection by a single aphid P
Exp. no.	1	5	10	20	
1	2	0	0	2	0·01
2	4	3	6	4	0·06
3	1	8	8	9	0·17
4	0	1	1	1	0·01
5	2	8	8	6	0·11
6	0	2	5	3	0·03
Total	9	22	28	25	—
Expected (susceptibility constant)	3·0	13·5	23·9	38·3	0·0496
Expected (susceptibility varying)	4·9	15·5	24·5	39·1	0·01–0·17

There is no good reason to doubt that the infections were local and independent. Neglecting the data from the 20 aphid groups, $p = 0{\cdot}0771$, $\pm 0{\cdot}0095$ with susceptibility constant and the expectations for the 1, 5 and 10 aphid groups are 5, 21 and 34 respectively; with $p = 0{\cdot}01 - 0{\cdot}20$, i.e. susceptibility varying, the expectations are 6, 20 and 30. The second of these distributions does not differ significantly from that observed ($\chi^2 = 2{\cdot}77$, $P = 0{\cdot}10 - 0{\cdot}05$). An even better fit is obtained if the anomalous results from Exp. 1 are rejected ($\chi^2 = 0{\cdot}989$, $P = 0{\cdot}5 - 0{\cdot}3$). The defection of the 20 aphid groups could be due to faulty technique because it was, on most occasions, found difficult to make so many aphids stay on a small plant.

The second experiment was designed to find whether age of infected leaf affected the transmission by aphids. Groups of 30 aphids were caged on five different leaves of a Norfolk Giant plant. The leaves chosen were the unfolding tip leaf, the next three leaves in basipetal succession and a senescing basal leaf. After 24 hr. infection feeding each group was moved in lots of three per plant to each of 10 *R. occidentalis* seedlings on which they spent a further 24 hr. The experiment was repeated four times between 15 and 28 August 1951, using one and the same Norfolk Giant plant for replicates 1 and 2 and a second plant for replicates 3 and 4. The results (Table 3) show that irrespective of plant of origin, young leaves are better sources of virus than older leaves, and are significantly better than senescing leaves. There is, however, a large error variance (S.E. = 27 % of mean for experiment), again suggesting variation in susceptibility among the healthy plants. The percentages of infection obtained were much higher than in either of the preceding experiments. This may be because small groups of *Amphorophora rubi*, an extremely active insect, settle down to feed quickly. Groups of 10 or more become almost unmanageable in warm weather because the aphids wander from the plants.

TABLE 3. *Number of plants infected by groups of three aphids fed for 24 hr. on infected leaves of different ages*

(Ten plants per treatment repeated on four occasions.)

	Feeding site					
	Tip leaf	Leaf 1	Leaf 2	Leaf 3	Basal leaf	Total
	8	9	1	0	2	20
	5	7	3	10	0	25
	10	7	9	4	2	32
	9	7	10	5	2	33
Total	32	30	23	19	6	110
Mean percentage infection	80	75	58	48	15	—

The apparently low vector efficiency of *A. rubi* suggested by Table 1 is therefore probably due in the main to the healthy plants resisting infection and to using too large groups of aphids.

Infectivity of aphids tested on successive healthy plants

Table 4 shows the results of an experiment designed to provide information on the time aphids remain infective. Groups of 10 aphids were given five different times of infection feed and then moved consecutively to two healthy plants. The time spent on the first plant was either, 1, 4 or 24 hr., whereas that on the second was constant, 24 hr. The data from the first plants are given in Table 1.

The number of second plants infected is correlated with the length of infection feeding time, but depends more closely on feeding time on the first healthy plant. Aphids infected fewer second plants when they spent 4 hr. instead of 1 hr. on the first plant and infected none at all when they spent 24 hr. on the first plant. These results were confirmed by a second experiment where groups of five aphids were given a 24 hr. infection feed and then moved consecutively to five healthy plants (Table 5). Infectivity declined rapidly after the first 2 hr. and was practically exhausted after 5 hr. feeding. The performance of each of the twenty-five aphid

TABLE 4. *Numbers of consecutive infections by groups of ten aphids after varying feeding times on both infected and healthy plants*

(Feeding time on second plants constant, 24 hr., ten plants per treatment, repeated on five occasions.)

I.F.P. (hr.)	Infections obtained on	T.F.P. on 1st plant (hr.)			Total
		1	4	24	
½	1st plant	2	2	2	6
	2nd plant	0	0	0	0
	Total	2	2	2	6
2	1st plant	25	16	21	62
	2nd plant	5	1	0	6
	Total	30	17	21	68
6	1st plant	21	17	25	63
	2nd plant	20	4	0	24
	Total	41	21	25	87
10	1st plant	12	21	27	60
	2nd plant	8	0	0	8
	Total	20	21	27	68
24	1st plant	23	19	16	58
	2nd plant	18	3	0	21
	Total	41	22	16	79
Total	1st plant	83	75	91	249
	2nd plant	51	8	0	59
	Total	134	83	91	308

TABLE 5. *Numbers of infections by groups of five aphids on five consecutive plants after 24 hr. infection feeding*

(Five plants per treatment repeated on five occasions.)

	Plant order (T.F.P.)					
	1st (1 hr.)	2nd (1 hr.)	3rd (1 hr.)	4th (2 hr.)	5th (24 hr.)	Total
	3	2	1	0	0	6
	2	0	0	1	0	3
	5	2	0	0	0	7
	4	3	1	2	2	12
	5	5	3	4	1	18
Total	19	12	5	7	3	46
Mean percentage infection	76	48	20	28	12	—

groups was separately recorded in this experiment. Three caused no infections on any occasion; Table 6 shows the distribution of infections caused by the remaining twenty-two. 65 % of the total number of infections and all the aphid groups that infected three or more plants are accounted for by two of the five replicates of the experiment. All the remaining aphid groups caused single or intermittent infections more frequently than consecutive ones. Such apparent differences in efficiency between aphid groups could result from variations in size of dose of virus required to cause infection and, hence, from variation in susceptibility of plants to infection. This would tend to exaggerate any real differences in vector efficiency between different groups.

TABLE 6. *Distribution of infections* (+) *caused by twenty-two groups of five aphids on five consecutive plants*

(Data from Table 5.)

	Plant order (T.F.P.)				
No. aphid groups	1st (1 hr.)	2nd (1 hr.)	3rd (1 hr.)	4th (2 hr.)	5th (24 hr.)
4	+	+	+	+	o
1	+	+	o	+	+
1	+	+	o	o	+
1	+	+	o	+	o
3	+	+	o	o	o
1	+	o	+	o	o
1	+	o	o	o	+
7	+	o	o	o	o
1	o	o	o	+	o
2	o	+	o	o	o
Total 22	19	12	5	7	3

CONCLUSIONS

The results of all these experiments are simply explained if aphids acquire increasing amounts of virus with increasing infection feeding time and that they rapidly lose virus. Young Norfolk Giant leaves are better sources of virus than older leaves and this may imply variation in virus concentration between leaves of different ages. But host plant resistance is the main factor limiting transmission by *A. rubi*.

The effects of starvation on the rates of development and loss of infectivity of *A. rubi* need further investigation. Because very few aphids transmitted virus after infection feeds of less than half an hour it seems unlikely that transmission of leaf mottle is increased by starving the vector as is the transmission of, for example, cucumber mosaic and potato virus *Y*. At least two other viruses, vein-banding and a latent virus of New Zealand Lloyd George are known to have much the same relations with *A. rubi* as leaf mottle (Cadman unpublished). With none of these viruses is infectivity subject to any delay in development. Differential aphid-feeding treatments are thus unlikely to offer an easy means of separating such viruses from one another.

It is a pleasure to thank Mr R. J. Rose for assistance with many of the experiments; Dr A. W. S. Hunter, Ottawa, for keeping me supplied with seeds of *Rubus occidentalis*; and Mrs M. A. Watson for her interest and help.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Meetings of the Association were held on 2 October (London) and 13 November (Bristol). The full list of speakers, with titles of papers, will be given in the Report of the Council of the Association.

The following is presented below, in slightly abridged form:

Paper read on 13 *November* 1953

Recent applications of growth substances in fruit growing. By Mr D. L. Abbott.

Also included, by permission of the Biological Council, is a contribution by Dr W. P. K. Findlay on the presentation of spoken papers:

Notes for speakers. By W. P. K. Findlay.

RECENT APPLICATIONS OF GROWTH SUBSTANCES IN FRUIT GROWING

By D. L. Abbott
Long Ashton Research Station, University of Bristol

(With 1 Text-figure)

Growth substances have now been used for a variety of purpose on many fruits for a considerable number of years. I want, however, to confine this talk to two applications of these substances to the growing of apples which are now under investigation at Long Ashton. The first, the application of growth substances as fruit-thinning agents, though not by any means new, is still very much in the experimental stage. In fact it is becoming something of a problem child. The second, the use of a growth substance as a fruit-ripening agent is comparatively recent and shows great promise of commercial application in the near future.

The first part of this paper, then, resolves itself into a consideration of the problems involved in developing the use of these substances. Naphthalene-acetic acid (NAA) has been used since 1940 as a pre-harvest drop spray; that is, it is sprayed on to the tree shortly before harvest to prevent the premature shedding of fruit. Its effect is to retard the formation of the abscission layer at the base of the fruit stalk (Barlow, 1950) and so cause the fruit to remain attached to the spur. NAA was therefore next applied early in the season in an attempt to reduce the June drop, the process occurring some 5–6 weeks after full bloom when the tree naturally sheds a large proportion of its young developing fruitlets. Contrary to expectations the application of NAA at this period usually had the opposite effect and resulted in an increased drop and consequent reduction of crop (Burkholder & McCown, 1941). The importance of this unexpected finding was quickly realized, for an efficient chemical thinning agent would be of tremendous value to the grower in cutting labour costs of hand thinning those varieties which normally carry a heavy crop of undersized fruit and in regulating the bearing of those biennial varieties which carry a heavy crop in the 'on' year and little or no crop in the following 'off' year.

Fruit thinning

Chemical thinning agents had previously been developed, but these depended upon the caustic action of various di-nitro compounds or covering the stigmas with oil-wax emulsions and so preventing pollination. Both these types of spray have to be applied at or about blossom time and this is often a disadvantage because the set, and consequently the degree of thinning required, cannot be estimated at this stage. Secondly, late frosts are not an uncommon occurrence in this country and it is a bad thing to thin chemically only to find that a subsequent frost destroys a further proportion of those fruitlets remaining on the tree. And thirdly, the foliage is young and delicate at this stage and very susceptible to spray damage.

Since the first observation of the thinning effect of NAA in 1941, a considerable amount of research has been carried out in many countries to develop it as a fruit-thinning agent, but results have generally been variable and often disappointing. Workers in the U.S.A. have frequently reported excellent thinning and resultant improvement of the crop and, in some districts, the use of NAA is reported now to be standard orchard practice (Murneek & Teubner, 1953). Encouraging reports have also come from Switzerland, but in this country, and in Holland, there has been little, if any, real success so far. Certainly we can thin the fruit and, as a result, usually increase the mean fruit weight, but this is not necessarily sufficient. Clearly, in order to be of commercial value, the thinning must be accompanied by a sufficient increase in fruit size to raise the cash value of the crop. For instance, a tree might bear three apples each worth 1*d*. Application of NAA might thin to two apples with an increased fruit weight, worth 1½*d*. each—the crop is still worth 3*d*. In order to be profitable, NAA must thin to two apples and increase their weight sufficiently to raise their value to 2*d*. each. Either this must be done, or, in biennial varieties, the fruit must be thinned in the 'on' year to such an extent that the tree crops again in the following 'off' year, the two crops together having as high or higher cash value than that of the single 'on' year crop of an untreated tree. These are the tests of a thinning agent. Can they be done with NAA and done with certainty?

Our experience at Long Ashton is not very encouraging. We have sprayed many varieties over a period of times and a range of concentrations. We find that NAA is effective as a thinning agent up to about 3 weeks after petal-fall, but the earlier the treatment the greater the effect of a given concentration. Similarly, the earlier the treatment, the greater, usually, is the resulting increase in mean fruit weight and, indeed, the later applications may even result in a decreased mean fruit weight. On the other hand, early applications, about the time of full-bloom, often cause foliage damage, as already mentioned, which may offset the benefit of thinning. Also remember that the advantage of NAA over the other types of chemical thinning agents is the fact that it can be used as a post-blossom spray. By field experiments we have tentatively reached the conclusion that application at about 7 days after petal-fall is a happy compromise between these opposing trends.

We have found, too, that the relationship between concentration and effect is approximately logarithmic, the higher concentrations, within a limit, causing the greater thinning and greater increase in mean fruit weight. However, on grading the fruit, we have not yet been able to obtain a greater absolute number of marketable fruit, but only an increase in the proportion of marketable/unmarketable fruit (Table 1).

Further, we have not always been able to reproduce results another year. Table 2 shows that whereas considerable thinning and resultant increase in mean fruit weight followed treatment in 1950, there was no significant effect in 1952. It is of interest to note that the mean fruit weight of the control branches was only 39 g. in 1950, but 54 g. in 1952, and it was suggested (Abbott, 1953) that the difference in response to NAA may have been due to an improvement in the nutritional status of the trees as a result of thinning alternate trees in the winter of 1951.

Other growth substances have been tried as thinning agents, but none has shown any more promise than NAA (Luckwill, 1952). Nor, so far, is there much evidence of the ability

TABLE 1. *Distribution of Crawley Beauty into size groups following treatment 7 days after petal-fall*

Treatment (p.p.m. NAA)	<1¾ in.	1¾–2 in.	2–2¼ in.	2¼–2½ in.	>2½ in.	Total Unmarketable <2¼ in.	Total Marketable >2¼ in.
Control	3	14	19	19	7	36	26
5	1	4	7	10	6	12	16
10	0	2	6	12	5	8	17
15	0	2	4	8	5	6	13
20	1	2	3	6	5	6	11
30	0	0	1	5	6	1	11

TABLE 2. *Effect of NAA at 20 p.p.m. applied 7 days after petal-fall on Miller's Seedling*

		No. fruit/100 blossom clusters	Mean fruit weight (gm.)
1950	Control	61	39
	Treated	27	51
1952	Control	65	54
	Treated	52	56

of NAA thinning sprays to regulate biennial bearing. Claims of increased fruit bud formation following the application of NAA in the 'on' year have been made from time to time, but no such effects have yet been observed at Long Ashton. This might be because we use branches and not whole trees as experimental units. A proper evaluation of the use of thinning sprays to regulate biennial bearing must await the establishment of sufficient new experimental trees in the near future.

The picture I have painted so far is rather gloomy. Clearly many factors are involved. If there exists, as indeed there must, a set of conditions, both internal and external, under which this spray does work, what are these conditions? This spray could be of such value to the grower that it cannot be discarded without the fullest possible investigation. Though data from further field trials are useful, it is only from a deeper understanding of the mechanism by which NAA brings about its thinning effect that progress is likely to be made. It is to this end that work of a somewhat more fundamental nature is now being carried out.

Some progress has already been made. We have already noted that one effect of NAA is to retard the formation of the abscission layer at the base of the fruit stalk. This is the effect made use of when NAA is applied as a pre-harvest drop spray. Luckwill (1953) has shown that this abscission layer is in nature normally prevented from forming because of the production by the developing seed of one or more natural hormones. Furthermore, he has demonstrated that the application of NAA as a post-blossom spray causes seed abortion. The result, then, of spraying the tree is temporarily to reduce the drop, due to the sticking-on effect of NAA and, at the same time, to kill the seeds and thus stop the natural flow of hormones. When, therefore, the first effect wears off, there occurs a greatly increased drop of all those fruits in which the seeds have been killed and from which, therefore, there is no longer a flow of natural hormone. This, incidentally, also explains the apparent anomaly of the same substance being used both to stick fruit on and pull it off.

We also know, from hand-painting experiments, that NAA exerts its influence through the leaves rather than by absorption through the fruit.

It was noted in Table 2 that very different results were obtained with Miller's Seedling in two seasons, and it was suggested that this might have been due to a change in the nutritional status of the trees following thinning. To obtain more information on this point an experiment was designed this year in which the relative nutritional status of branches of Laxton's Superb was artificially raised by hand thinning prior to spraying with NAA. Two methods of hand thinning were employed; in the one four of every five blossom clusters were removed and in the other four of every five flowers were removed. Since each cluster on average contained five flowers, this amounted to thinning each cluster to one flower. The extent of thinning was thus the same in both methods, i.e. a reduction to one-fifth the original number of flowers. The hand thinning was done at full-bloom and NAA was then applied to half the branches at the usual 7 days after petal-fall. No further thinning, resulting from the spray, was observed on those branches on which the flowers had been reduced to one per cluster, while on those on which the clusters had been reduced, treatment with NAA resulted in approximately the same degree of thinning as on the control branches. This suggests that NAA is not directly toxic to the seed since the greatest concentration per fruit must have occurred in those fruit thinned to one per cluster. Rather does it suggest that the action of NAA is to accentuate the deficiency of some factor required by the developing seed and already in short supply due to competition between the fruitlets on an individual spur. This is in accord with our experience with Miller's Seedling and with the observations of other workers in this field that weak spurs are more easily thinned than vigorous spurs (Langer, 1953).

The picture so far is, then, that the spray is absorbed by the leaf and there, or in some other part of the plant, aggravates an existing deficiency of some factors to such an extent that the seeds of many fruitlets are killed and the fruitlets fall. The problem is to determine the time, concentration and conditions in which to apply the spray to cause maximum *temporary* shortage of this factor.

The next step is to determine the nature of this factor. Is it fabricated food material whose production is slowed down by interference with the photosynthetic mechanism, or whose translocation is disrupted? Is water in short supply due perhaps to excessive transpiration following application of NAA? Maybe some natural hormone or precursor manufactured by the leaf and required by the seed is destroyed or inhibited by NAA. These, and the effects of light, temperature and other external factors upon the spray, are some of the questions which must be answered before we can hope to make NAA or any other substance work as a thinning spray to the best advantage and with certainty from year to year.

Acceleration of ripening

Let me turn now to the second application of growth substances to fruit growing—this is the use of 2:4:5-trichlorophenoxy propionic acid (2:4:5-TP) as a ripening agent. In a search for more efficient pre-harvest drop sprays, it was noticed that many of the substances tried caused an earlier development of colour and an apparent hastening of maturity. Of the substances tested, 2:4:5-TP seemed to have the greatest activity in this respect (Edgerton & Hoffman, 1951; Luckwill, 1952), and so has been further investigated for two reasons. First, and most obviously, there is always a demand for apples early in the season and therefore the earlier the fruit can be marketed the better the price obtained. Secondly a grower with a large acreage of any variety is faced with the difficulty of having to harvest them all within a very limited period. One method of overcoming this difficulty is to apply a pre-harvest drop spray and so prolong the picking period. A ripening spray applied to part of his orchard would enable the grower to advance his picking date and so prolong the harvest period even further.

Some information on this new use of a growth substance was obtained last year in a pilot experiment in which branches of Worcester Pearmain were sprayed with 2:4:5-TP. This variety was selected because it is widely grown in this country, it is an early apple and the fruit is naturally highly coloured. The spray, at 10, 20, 50 and 100 p.p.m. was applied on 11 June, 9 and 28 July, that is 13, 9 and 6 weeks before the normal picking date. 100 p.p.m. was obviously too high a concentration. It caused the fruit to colour very much too quickly and before any flavour had developed. The fruit was of poor quality and tended to break down with rot around the calyx. In addition, it caused severe foliage damage and die back of the shoot tips. Similar effects, though far less marked, also occurred after spraying at 50 p.p.m.

Concentrations of 10 and 20 p.p.m. were too low and caused only very slight ripening.

The first application, that made 13 weeks before the normal picking date, was too early. The branches were sprayed before the end of the June drop and in consequence many fruits were held on the spurs which otherwise would have dropped. This resulted in a crop of excessive numbers and extremely small size. Spraying 9 weeks before normal picking date also resulted in rather small fruit since they were ripened and ready to harvest before they had grown to a reasonable size.

The limits were thus narrowed; it seemed that 2:4:5-TP should be used at a concentration of 50 p.p.m. or below, but higher than 20 p.p.m., and that the spray should be applied about 6 weeks before the normal picking date.

A further trial was made this summer in which whole trees of Worcester were sprayed at 30 and 50 p.p.m. on 15 July and 4 August, that is 8 and 5 weeks before normal picking date.

I must now digress for a moment and explain that one of the difficulties of this type of work is to measure ripeness. As a fruit becomes ripe many changes occur—the flesh softens, starch is converted to sugar, the acid content falls and many subtle chemical reactions take place which lead to the development of flavour as well as to changes in colour. These happenings are not necessarily correlated and no one test will give a measure of ripeness. The best one can do is to select a representative series of tests, and those that we have adopted at Long Ashton are, briefly, as follows:

(1) A pressure test in which the resistance of the flesh to a plunger is measured.

(2) Sugar content.

(3) Acid content in which the total titratable acidity is measured.

(4) Starch content. A half apple is painted with iodine solution and the area in which starch is present is estimated.

(5) Seed colour.

(6) Fruit colour is measured, but this again presents a problem since so many colours, shades and tones, are involved that no simple test such as matching against a colour chart will suffice. It was decided, in spite of the limitations of the method, that the only practicable solution was to extract the three main classes of pigments and measure these in a Spekker absorptiometer. These pigments are, broadly, the red pigments, anthocyanins, soluble in water; the green pigments, chlorophylls, soluble in ether and saponifiable, and the yellow pigments, xanthophylls, also soluble in ether but not saponifiable.

To return, then, to this year's trial, Fig. 1 shows the effect of the treatment upon anthocyanin content.

It is of interest to note that all treatments result in a higher anthocyanin content than the controls, and that the later the spray is applied, the greater is the eventual effect of a given concentration, for while 50 and 30 p.p.m. applied on 4 August resulted in the same anthocyanin content, 30 p.p.m. applied on 15 July resulted in an anthocyanin content only intermediate between 50 p.p.m. and the control. This intermediate anthocyanin content was due not to partially ripened apples, but to samples in which some fruit were ripe while others were still green, since this treatment resulted in very uneven ripening. It suggests that some critical concentration of 2:4:5-TP is required to trigger off the ripening mechanism.

This pattern is typical of the results obtained for the other tests, though there are, of course, many minor variations. Generally colour is most profoundly affected by treatment, but without doubt this substance does influence the whole metabolism of the fruit. It is apparent that saleable fruit of very attractive appearance and good size can be marketed as early as 3 weeks ahead of normal, but the flavour is not fully developed and even a few more days on the tree would benefit the size. Provisionally it seems that treatment at 40 p.p.m. 6 or 7 weeks before the normal picking date is likely to give the optimum effect.

A preliminary test was made to determine the effect of treatment with 2:4:5-TP on the storage quality of the fruit. As might be expected, this is impaired by the treatment. By the middle of October the control fruit was still sound, fruit sprayed on 4 August was fast nearing the end of its storage life while fruit sprayed on 15 July was wrinkled and no longer saleable. Though this is not likely to be a disadvantage with an early variety like Worcester, which can be put into the shops as soon as harvested, it may prove troublesome if used on later varieties which may subsequently have to be stored. It must also be remembered that 2:4:5-TP is a pre-harvest drop spray as well as a ripening agent and the tendency to leave the treated fruit on the tree too long must be avoided.

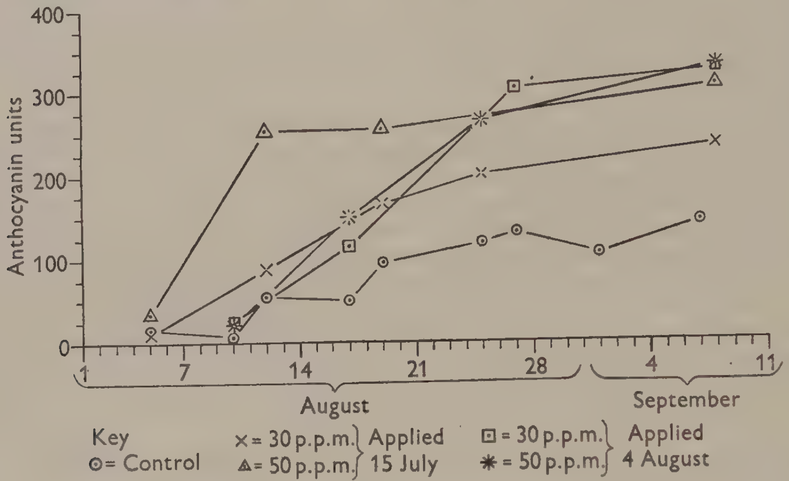

Fig. 1. Effect of 2:4:5-TP on anthocyanin content of fruit.

To finish with a word of caution, I would point out that we have not yet seen what will happen to these trees next year!

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NOTES FOR SPEAKERS

Many papers are read at meetings of scientific societies by speakers who have had little platform experience, and often the presentation of these papers is not good enough to arouse and retain the interest of the audience, so that accounts of fundamentally interesting work frequently do not receive the attention that they merit.

The following notes have been prepared by the Biological Council in order to assist speakers at scientific meetings in the same way as 'Instructions to Contributors' are issued to guide authors contributing papers to scientific journals. They are intended to draw attention only to the basic essentials of public speaking, which is an art that must be learnt. Too many people are content with a standard of delivery far lower than they could achieve if they were to give a little thought to the practice of the art.

Preparation of the paper

The paper that has been prepared for publication in a scientific journal is seldom completely suitable for verbatim presentation at a meeting: vice versa, the most successful spoken exposition cannot normally form the text of a paper for publication in a journal, because spoken presentation of ideas to an audience and the written presentation of the same ideas to a reader usually require a different phraseology. Data that may appear in tabular form when printed can often be better presented to an audience in the form of graphs or histograms.

The paper should *never* exceed in length the time allotted to it by the Secretary. Nothing is more embarrassing to the speaker, more irritating to the Chairman, or more unfair to fellow speakers, than a paper that exceeds its allotted span of time. A paper should be rehearsed with a watch and at least 10% margin of time allowed for incidental comments and disturbances. If slides are to be used plenty of time must be allowed for their presentation and explanation.

The introduction to the subject should explain as briefly as possible the background and purpose of the investigation, but any survey of historical literature should be kept to an absolute minimum. All too often speakers waste half their precious time surveying earlier work. A logical theme should be followed in presenting the story of the work—not necessarily the chronological sequence of the experimental work. Descriptions of methods should be reduced to a minimum unless they are really novel. Only those results should be presented that are essential to justify the conclusions that are to follow. What interests the audience is the discussion of the results and their theoretical implications. Lengthy tables of data should never be presented and no descriptions should be given of any structure which can better be presented in visual form.

Delivery of the paper

Only when a formal paper such as a Presidential Address is to be given should the contribution be read from a manuscript. However skilfully a paper is read, it can never have the fresh spontaneity of a spoken address. If a speaker feels unable to dispense with a full manuscript he should attempt to memorize at least the discussion and conclusions. By plentiful use of marginal headings in red ink, he can provide notes that will remind him of the main points in his story so that he can refer to the manuscript if he 'dries up'. Points at which slides have to be shown should be clearly marked on the lecturer's notes.

The speaker must be heard clearly even at the back of the hall. Nothing makes an audience lose interest more quickly than inaudibility of the speaker. Conversational tones are never adequate on the platform. Always direct the voice to the back of the room—speak 'over' rather than 'at' the audience. Every hall has different acoustic properties and the speaker must tune his voice to the hall. It is more difficult to speak in long low rooms than in shallow or round ones. If there is any doubt as to the carry of the voice a friend at the back of the room should be asked to signal instructions. Do not drop the voice at the end of a sentence and never drop the voice because you have come to a less important part of the paper. Repeat, preferably in slightly different words, any sentence that contains the gist of the argument or the final conclusion. Do not continue speaking when using the black-board and when a slide is on the screen speak only about what is there illustrated.

The speed of delivery should depend on the nature of the subject under discussion. If it is simple and familiar to the audience quite a brisk rate of speaking is appropriate. If it is unfamiliar a slow deliberate delivery should be used. Variation in the speed of delivery adds emphasis and colour to speech and should be used to highlight important phrases. It is much better to pause between phrases and sentences spoken at reasonably fast speed than to speak each word slowly. Nothing is more soporific than an absolutely steady, slow rate of delivery.

Enunciate with especial care technical words likely to be unfamiliar to some of the audience and write on the black-board the Latin or foreign names of any organisms that you mention, or any new words that you coin.

When addressing an audience containing a proportion of foreigners—as at any international congress—the rate of delivery should be about half that normally used.

Gesture should be used sparingly. Though it can be extremely effective when used skilfully, the inexperienced should remain still when speaking and avoid fidgeting with chalk etc. Any movements that distract the audience, such as pacing up and down like a caged lion, should be avoided.

Visual illustrations

Any diagrams used should be written in bold lettering clearly visible at a distance and should contain the absolute minimum of words. They should be firmly fixed to boards—how often do lecturers become involved in coils of paper when they unroll charts before an audience.

Tables on the black-board should be as brief as possible so that results are obvious without detailed explanation.

Lantern slides should always be shown in preference to using an epidiascope. If the latter has to be used see that the pictures are the right size to fit the screen. Give the audience time to appreciate the points illustrated in a slide. Never show a slide in which the desired detail is obscure. If the lettering on a slide is not easily legible to the naked eye at normal reading distance it is unlikely to be clearly visible when projected on to a screen in an average lecture theatre. It is better to show no slides than poor ones. Typed or printed matter seldom shows up well on a slide—a photograph of good lettering makes a better slide—and above all do not include too much material on a slide, otherwise none of it may be visible to those beyond the first few rows of seats.

Cinema films should be used only to illustrate moving subjects. Stationary objects can often be shown better by means of stills.

When displaying an object to the audience hold it up so that they can see it. Avoid passing round objects during a talk, for their passage from hand to hand distracts the audience from what is being said.

Summing up

1. Never exceed the time allotted for the paper.
2. At all costs ensure that your words are clearly heard by everyone in the room.
3. Integrate and co-ordinate the visual with the spoken.

W. P. K. FINDLAY

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REVIEWS

Endosymbiose der Tiere mit pflanzlichen Mikroorganismen. By Paul Buchner. (Lehrbücher und Monographien aus dem Gebiete der exakten Wissenschaften: Reihe der experimentellen Biologie, Band 12.) Sup. Roy. 8vo. Pp. 771 with 336 Text-figures and 3 Plates. Basel und Stuttgart: Verlag Birkhäuser. 1953. 66.50 Swiss francs; 66.50 D.M.; £5. 17*s*.

No worker has done more to advance, or so much to shape and organize, our knowledge of symbiosis as Paul Buchner, student of Boveri and Hertwig, and for many years Professor of Zoology in the University of Leipzig. Since 1911 memoirs, monographs, and books have streamed from his pen and now, in his 67th year, comes this *magnum opus*. It is the third time he has surveyed the entire field. In 1921 appeared his *Tier und Pflanze in intrazellularer Symbiose*, of which a greatly enlarged second edition was published in 1930 under the title *Tier und Pflanze in Symbiose*. In 1939 appeared his *Symbiose der Tiere mit pflanzlichen Mikroorganismen*, with a second edition ten years later. Experimental researches carried out during the last decade have given much new evidence and led to considerable re-interpretation of older knowledge, so that Prof. Buchner has modified or changed many of his views and orientations. Hence, the present volume is not merely a revised third edition of the previous book; it is essentially a new work, encyclopaedic within its terms of reference, and as definitive as any scientific work could hope to be in such a rapidly changing scene.

The book is divided into three parts: an introductory historical survey followed by special and general sections. In the first two chapters the author draws an admirable picture of the development of the idea of symbiosis, first of animals with algae (pp. 17–36), and then of animals with fungi and bacteria (pp. 36–73). The short third chapter (pp. 73–80) might well have been headed 'Achtung!' for it is a salutary warning to students of symbiosis regarding errors of interpretation, wayward paths and blind alleys.

The Special Section (pp. 81–538) forms the mass of the book. In seven chapters the author describes in detail the relations between animal hosts and their microbial endosymbionts: in ambrosia insects and others that feed on cellulosic materials, eg. plant parts, seeds, etc. (pp. 81–189); in insects that live in plant exudations (pp. 189–92); that suck plant juices (pp. 192–371); that suck vertebrate blood or devour horny substances (pp. 371–444); that feed on a mixed diet (pp. 445–70); in luminescent animals (pp. 470–528); and symbioses localized in excretory organs (pp. 528–38). Prof. Buchner ranges critically and impartially over the entire field, and in these 458 pages deals not only with symbioses in insects where they are, perhaps, best known, but also with those in annelids, cephalopods, tunicates, fishes, etc. Taxonomic arrangement within each chapter, which amounts to arrangement by types of symbioses, involves slight duplication but enables the author to systematize the enormous mass of information in clear and simple fashion. In each type or case he describes the morphology and taxonomy of the microbial symbiont, its entrance, passage, localization and transmission, and the structure and development of the host tissues or organs involved. There can be few fields of biological research in which the described 'facts' range so obviously from instances of casual association to complex, obligatory and specific relationships, and where published accounts range from naïve wishful thinking to such critical studies as those, for example, of H. J. Müller on the Homoptera. Prof. Buchner's treatment of his subject in this part of the volume is 'German scholarship' at its best: encyclopaedic consideration, meticulous attention to detail, and a passionate integrity in the setting out and weighing of evidence.

The last part of the book, the General Section, comprises six chapters in which are discussed in great detail the location of symbionts (pp. 539–58); transmission of symbionts (pp. 558–75); embryonic and post-embryonic events (pp. 575–97); reciprocal relations between hosts and symbionts (pp. 597–640); evolutionary problems of symbiosis (pp. 640–72); and, finally, the significance of symbiosis (pp. 672–725). These 187 large pages of very solid print, lightened only by ten text-figures and five tables, contain scientific writing of high quality. The problems the author is dealing with are all complex, involving many abstruse and variable factors. Prof. Buchner examines this mass of conjecture and fact with patience, insight, and a rare impartiality, and the outcome is a coherent and convincing narrative. Fortunately, the experimental researches of the last few years, based largely on the pure culture of symbionts, the sterile culture of hosts, and the bringing together of the two or more components under known or controlled conditions of environment and particular developmental phases of host and symbiont have thrown much light upon the symbiotic state. In little more than a decade the whole front line of research has moved forward. From the secure morphological base established by earlier workers, a successful advance has penetrated wide physiological territories, and salients have been driven deep into fields of animal behaviour, of nutrition and metabolism, enzymes, vitamins and growth substances, of biochemistry, and of microbiology, antibiotics and immunology. In making full use of this recent expansion of knowledge to elucidate his problems the author brings to bear a scholarly and wide-ranging mind which is sensitive in its discrimination and stored with a lifetime of rich experience.

But probably no biologist could agree with all the author's viewpoints and findings; a reader must often have doubts, misgivings and reservations which, I am quite sure, would meet with Prof. Buchner's approval and sympathy, for no one knows better than he how the experimental study of even the simplest problem of symbiosis leads by devious ways into obscure and still largely unexplored regions of animal and microbial biochemistry, physiology and behaviour. To-day there is comparatively little divergence of opinion regarding the descriptive morphology, localization, histology, etc., of host and symbiont; but there is lively controversy regarding problems of necessity and transmission, whilst the physiological significance and evolutionary origins and trends of the communal state are almost open questions. One's personal views depend largely not only upon one's idea of what constitutes symbiosis, the necessity and stability of this condition, and its relation to antibiosis, communalism, commensalism, and parasitism, but also upon one's approach to, and interpretation of, any given 'facts' about the communal life of two or more organisms. How wide is the scope for divergence on common 'facts' may be gauged by reading, for example, the contributions to the 1951 Amsterdam symposium on '*Symbiose chez les Insectes*', or by comparing Buchner's discussion of luminescent animals in the present volume with that by E. Newton Harvey in his *Bioluminescence* (1952). It seems clear to me that whilst every biologist interested in problems of symbiosis must gratefully accept Prof. Buchner as a wise and immensely knowledgeable guide through this labyrinth, he will be well advised to retain the utmost independence of mind and capacity to reserve judgement. In regard to perhaps the majority of these problems conclusions are just not yet possible, nor have they been brought any nearer by the experimental researches of the last decade. One can only remain agnostic until more definite and compelling evidence becomes available.

The book is well illustrated but, with one exception, all the text-figures are line drawings, many re-drawn from published drawings or photographs. This has the advantage of uniformity of illustration but, from time to time, one longs for some good photomicrographs. The three half-tone plates all illustrate microbial symbionts. The book opens with a synoptic contents of nine pages which, however, does not wholly compensate for the absence of a subject-index. It closes with a thoroughly international bibliography of nearly 1100 citations, some of the year 1952, an index of authors and a combined index of animal and plant names. Like all the previous volumes published by Verlag Birkhäuser in this Series the book is beautifully produced. WILLIAM B. BRIERLEY

Symposia of the Society for Experimental Biology. VII. Evolution. Ed. by R. BROWN and J. F. DANIELLI. Pp. xix + 448. Cambridge University Press. 1953. 45*s.*

This volume contains the papers read to a joint meeting of the Society for Experimental Biology and the Genetical Society held at Oxford in July, 1952. The volume opens with a foreword by J. B. S. Haldane who 'pin-points' the contributions and suggests some of their implications, and closes with a Postscript by J. F. Danielli on 'Some physical and chemical aspects of evolution'. There are plate and text-figure illustrations, and each paper ends with a list of References.

At first glance the field appears so vast, and the contributions so uneven and disparate, that one wonders if the book is anything more than twenty-three *ad hoc* papers on evolutionary topics, especially as each writer confines himself to his own problem without cross-reference, and there is no index. But then the volume does not set out to be a text-book of evolution: for that we have Julian Huxley's *Evolution, the Modern Synthesis* or any one of a dozen recent volumes by Cuénot, Dobzhansky, Mayr, Rensch, Schmalhausen, Simpson, Stebbins, etc. This book is for browsing and quiet meditation; it is a treasury of ideas, of orientations, approaches and viewpoints, of relationships and trends. The direction and emphasis of its contents are, of course, largely due to the fact that the meeting was one of experimental biologists and geneticists. But there is more to it than this. The vital advances, the new orientations and approaches, are in fact being made by just these groups of workers and not by the descriptive morphologists, comparative anatomists and embryologists, museum taxonomists, palaeontologists, and other students of those branches of science which, a generation ago, were almost universally accepted as the natural fields of evolutionary study. 'After all', as Baldwin says, 'evolution is still going on and mutation is still a daily occurrence' but now the problem is not what is evolving but how it does it.

Thus, of recent years, speculation about the origin of life has become almost respectable and finds support in cosmogony, geochemistry and thermodynamics. Whilst there is retained the 'holistic' view of the evolving organism characterized by a norm of reaction to its surroundings, the evolution of morphology has been transmuted into the biochemical or biophysical evolution of genes and genic systems. Adaptation remains, of course, a central problem but its genetical study in viruses, micro-organisms, and other populations now involves not only ecology but ethology. Speciation has become almost a physiologic-genetic concept, with the dynamics of evolution seen in terms of polymorphism or polyphasy, 'polyethism', selective differentials and intensities, isolating mechanisms, racial specialization, hybridism, and the ecological and biochemical adaptability or plasticity of segregates. In to-day's evolutionary perspective odour, movement, behaviour, habit, and social structure and patterning, are as significant as morphology and embryology and are visualized in terms of function and comparative physiology. And all these modern orientations and trends centre in and about that nexus formed by the coalescence of microbiology, physical chemistry and biochemistry, physiology, ecology, and cytogenetics, with statistics acting throughout as 'mentor'. Seen thus, evolution is a thrilling field of experimentation and philosophy which is being cultivated with great speed and considerable success, and I can think of no other single volume that brings this modern outlook so vividly to one's mind in so stimulating a fashion.

WILLIAM B. BRIERLEY

Tea Pests and Diseases and their Control: with Special Reference to North East India. By ERNEST HAINSWORTH. Pp. xi + 130, with 32 plates: Cambridge: W. Heffer and Sons Ltd. 1952. 18*s.*

The tea industry of north-east India—in Assam, west Bengal and east Pakistan—has been served for the past half century by the Indian Tea Association's Tocklai Experimental Station, one of the first of all 'commodity' research centres. The author of the book under review was Plant Pathologist at Tocklai for a number of years subsequent to the war, and he duly acknowledges his indebtedness to his predecessors and former colleagues at the Station. He introduces the book as a 'practical guide to the tea planter', to assist in the recognition of the various pests and diseases and, in particular, 'to enable a distinction to be made between the economically important and the unimportant, so that a decision may always be reached as to whether to apply control measures or to do nothing'. This purpose has undoubtedly been fulfilled; and, as in the Foreword contributed by Dr H. H. Mann (co-author, with the late Sir George Watt, of *Pests and Blights of the Tea Plant*, published in 1903), the author is to be congratulated on 'the very clear and practical account of the pests and diseases of one of the most interesting crops in the world'.

The arrangement of the subject-matter is mainly according to the parts of the plant affected, or the stages in its agricultural life history. The chapter on root diseases, to which particular attention is directed by the author in his Introduction, deals principally with *Ustulina zonata*, and with attack by *Sphaerostilbe repens* in relation to water-logging. Of stem diseases, the dieback associated with various *Nectria* spp. is described as almost invariably a consequence of hail damage, to be prevented by a protective spraying with a copper fungicide within twenty-four hours of such damage occurring. The chapter dealing with diseases of the leafy shoot draws attention to the fact that blister blight (*Exobasidium vexans*) has apparently become much more widespread in north-east India during the same post-war period that has seen its extension also to south India, Ceylon, and the East Indies. The short account of 'rim blight' ('marginal necrosis') relates this condition to possible derangements of calcium metabolism; but the reviewer must categorically deny having described similar symptoms in association with the 'phloem necrosis' virus disease in Ceylon (p. 71). Red spider (*Metatetranychus bioculatus*) is described as the most serious pest of the leafy shoots; its control is effected by the provision of shade and other cultural measures, by defoliation during the winter months, and by spraying or dusting with sulphur as the bushes come into bearing. There is a chapter on the 'Common Calamities' of drought, earthquake, hail, lightning, fire, and floods; and three chapters on control measures in general—on equipment (including a note on 'Spraying Developments in Ceylon' by the Director of the Tea Research Institute of that country), on materials, and on the practical items of estimates, working plans, and the recommended spraying calendar. The book is illustrated by twenty-four photographs, and by eight photographic reproductions after du Pasquier (1933); Plates 2, 10 and 14 are not particularly clear (Plate 10 is also quite irrelevant to the text), but the others are excellent and well chosen. The indexing is good. There are a few misprints, of which the variant spellings of the green manure species *Crotalaria anagyroides* and *Tephrosia vogelii*, and the reference to the late T. E. Petch (*sic*) in the author index and on p. 54, are among the most noticeable.

The book will be of general interest as a factual account, and assessment, of the diseases and pests of one of the major plantation crops, in one of the principal areas of its cultivation. These areas are relatively remote from one another and they invite comparison from the plant pathological viewpoint. To the reviewer, as familiar with conditions in Ceylon, the relative importance of *Ustulina zonata* as compared with *Poria hypolateritia*, and the status accorded to '*Nectria*', shot-hole borer, '*Cercosporella*', and tea tortrix, in north-east India, are outstanding examples of the extent to which the problems of these two areas differ. The organization of estate labour for pest and disease control purposes is another matter, for here the problem is essentially the same wherever the crop is grown. The treatment of this aspect is convincing and realistic; it is one of the best features of the book. T. E. T. BOND

Wine Growing in England. By GEORGE ORDISH. Pp. 128. London: Rupert Hart-Davis. 1953. 7*s*. 6*d*.

The last few years have seen a widening interest in amateur winemaking and in the growing of out-door grapes. This handbook gives a concise and highly readable account of the whole process from the selection and planting of the vines to the bottling of the wine itself. Being written by an enthusiast, the book should make many converts. An introductory section deals with the history of wine growing in England. This is followed by some twenty pages on growing the grapes, in the open and on walls, and the remaining pages are given mainly to the principles of wine-making and to the directions for making wines of different types including champagne. A chapter on other fruit wines is also included. The procedure is illustrated by tables and by photographs and diagrams: simple methods of chemical analysis are given in an appendix.

Books on this subject have sometimes been criticized as being either too chemical or of savouring of alchemy; Mr Ordish has successfully steered the mean path between these extremes. It is a pity, however, that he has kept in circulation some microbiological nomenclature that has outlived its day. The methods that he describes should be easily followed by the layman, but in the opinion of the reviewer, some of the levels of sulphur dioxide recommended may give rise to difficulty in inexpert hands.

The last chapter of the book is of particular interest, being devoted to the possibilities of commercial vine growing and wine production. The figures given are admittedly tentative but they provide a useful basis for the prospective grower.

A. POLLARD

Bibliographie der Pflanzenschutz-Litteratur 1940–1945. Edited by J. BÄRNER. 2 vols. Pp. xlviii + 1308. Berlin: Paul Parey. 1953. (No price stated.)

Those who profited from Morstatt's bibliographies of literature on plant diseases and pests, which appeared annually between the two world wars, will welcome the re-issue of the series from the Biologische Zentralanstalt für Land- und Forstwirtschaft in Berlin-Dahlem after a lapse of ten years. This has been made possible from funds provided by the United States under the European Recovery Programme.

These two volumes list the world literature on all aspects of plant diseases and pests published during the six-year period 1940–45, and they are to be followed shortly by another one covering the years 1946–51. It is easy enough to trace in them the publications of a particular person, for there is a good index of authors, but for those unfamiliar with the earlier issues it requires a little patience and practice to track down the literature on the diseases and pests of a specific crop or plant, or to find the publications on a particular family of fungi or insects. The list of contents, which like the introduction and chapter headings is given in German, English and French, is an elaborate one and provides the clues in searching for subject literature. There are over 25,000 entries.

W. C. MOORE

How Animals Move. By JAMES GRAY. Pp. xii + 114. Cambridge University Press. 1953. 16*s*.

There can have been few more successful Christmas lectures to the Royal Institution than those by Prof. Gray on 'How Animals Move', illustrated as they were by an entertaining and vivid series of demonstrations on living animals and on mechanical models. This book is based on the substance of the lectures and preserves in a remarkable way the easy and absorbing style of the original. Starting from an elementary treatment of the mechanics of propulsion, it goes on to describe swimming, walking and running, jumping and creeping, and finally flying; while the main emphasis is on the locomotory mechanism, the problems of control and energy supply are clearly indicated and the relationship between the anatomy and mode of life is everywhere emphasized. The illustrations are carefully designed to elucidate the text and the sequence of ideas can be followed by anyone who has (say) a background of School Certificate mathematics. Because of its attractive presentation, the book can, and doubtless often will, be read through at a single sitting—but it will well repay further study by the naturalist, as well as by the anatomist or physiologist. JOHN E. HARRIS

Nature and Structure of Collagen. Papers presented for a discussion convened by the Colloid and Biophysics Committee of the Faraday Society. Edited by J. T. RANDALL, F.R.S., assisted by S. FITTON JACKSON. Pp. 269. London: Butterworths Scientific Publications. 1953. 42*s*.

The symposium on the 'Nature and Structure of Collagen' gathered together scientists working in many different fields, and this report of the 24 papers given is a most welcome collection of information. Had a title been necessary for this review I would have chosen 'From Leather to the Limelight' for that is what has happened to collagen in the last decade or so. Part of the extended interest in collagen now stems from medicine, and this aspect is hardly touched on but the chemical analysis and physical properties of collagens from different sources and from animals of different ages are fully dealt with.

The papers show that there is great diversity in proteins now termed collagen. The most constant feature seems to be the high angle X-ray diffraction pattern; the banded structure shown in the electronmicrograph (EM) and the amino-acid analysis being far more variable. Fitton Jackson & Randall show that when collagen is precipitated from solution the E.M. striation depends on the pH and on the molarity of the precipitating salt. A further paper by Randall and his collaborators describes the byssus threads of *Mytilus edulis* which show a typical collagen X-ray diagram but no E.M. striation. A comparison of the amino-acid analysis of different collagens shows that collagen of rat tail tendon differs from that of ox hide and both from that of fish or of *M. edulis*. A method of quantitative paper chromatography, on which these analyses are based, is described by Brown, Kelly & Watson.

The early work of Nageotte in France showed that collagen from some sources could be brought into solution in dilute acid. Orekhovitch, working in the U.S.S.R., has isolated from skin a separate soluble collagen which he has named procollagen. This work has brought to the fore the relation between collagen and soluble skin proteins and Harkness, Marko, Muir & Neuberger, in a very interesting paper, describe the extraction of three separate collagen fractions from skin: alkali soluble, acid soluble and insoluble collagen. They have shown, by tracer methods, that the rate of turnover of alkali soluble collagen is far greater than that of the other types, and suggest that this fraction may be a precursor of the insoluble collagen.

There are two papers only on reticulin, the substance which histologically appears to be the precursor of collagen. Kramer & Little have prepared it from kidney using as starting material the connective tissue stroma of the cortex which is free from collagen. They confirmed the absence of collagen and other fibrous proteins histologically. Such preparations examined in the electronmicroscope showed a membranous structure in which fibres lay in an amorphous ground substance. The fibres showed cross striations typical of collagen. Cruikshank & Hill describe the histochemical identification of a connective tissue antigen from renal cortex. This antigen is absent from collagen but is present in basement membrane and reticulin and may contain a polysaccharide. Both basement membrane and reticulin stain strongly with the periodic acid Schiff technique.

Prof. Randall, who organized the symposium and has edited the report, is to be congratulated. The subject is in a healthy state of complexity, and the work going on at present should lead to understanding of the relation of collagen to carbohydrates and to other fibrous proteins. One can only regret that at 42*s*. the book is probably too expensive to be widely bought. A. PIRIE

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THE DISTRIBUTION OF VIRUSES IN DIFFERENT LEAF TISSUES AND ITS INFLUENCE ON VIRUS TRANSMISSION BY APHIDS

By F. C. BAWDEN, BRENDA M. G. HAMLYN and MARION A. WATSON

Rothamsted Experimental Station, Harpenden, Herts

Exposing both surfaces of leaves systemically infected with cabbage black ring spot virus (CBRSV) or henbane mosaic virus to ultra-violet radiation decreases the infectivity of expressed sap to about one-fifth. As irradiation probably inactivates virus mainly in the epidermis, which occupies about one-quarter the volume of the leaves, these viruses seem to occur at much higher concentrations in sap from the epidermis than in sap from other cells. By contrast, tobacco mosaic virus seems not to occur predominantly in the epidermis.

CBRSV and henbane mosaic virus are normally transmitted most frequently by previously fasted aphids that feed for only short periods on infected leaves, but aphids treated like this transmit rarely from leaves that have been exposed to ultraviolet radiation. Irradiation has relatively little effect on the proportion of aphids that transmit after long infection feedings. Fasting seems to increase transmission by increasing the probability that aphids will imbibe sap from the epidermis of leaves they newly colonize. With longer periods on infected leaves, the ability of fasted aphids to transmit probably decreases because they then feed from deeper cells and their stylets contain sap with less virus. Only virus contained in the stylets seems to be transmitted, not virus taken into the stomach. About half the transmissions of henbane mosaic virus by aphids that have colonized tobacco leaves for hours may be caused by insects that temporarily cease feeding on the phloem and newly penetrate the epidermis.

Irradiating infected leaves affected the transmission of sugar-beet mosaic virus in the same way as that of henbane mosaic virus, but had little effect on the transmission of beet yellows virus, whose vectors become more likely to transmit the longer they feed on infected plants.

Watson (1936, 1938) found that the proportion of *Myzus persicae* (Sulz.) that transmitted henbane mosaic virus (then known as Hyoscyamus virus III) after feeding for a few minutes on an infected leaf, was considerably increased if the aphids fasted for some time immediately before they were placed on the infected leaf. The response to fasting occurred only when aphids fed on infected leaves for short periods, and prolonging the infection-feeding times decreased the proportion of fasted aphids that transmitted the virus to healthy plants; with infection-feeding times of 1 hr. or more, fasted and non-fasted aphids produced similar proportions of infective individuals. Since the original work with henbane mosaic virus, many other viruses whose vectors cease to be infective within minutes or hours of leaving infected plants have been shown to behave similarly.

Various explanations for the phenomena have been advanced, but there has been no conclusive evidence in favour of any. Watson (1936) suggested that the increased transmissions with short feeding periods might occur because the superficial tissues then being penetrated by the aphids' stylets contained more virus than the deeper tissues penetrated later. However, as only fasted aphids responded to a short infection feeding, she did not favour this view and suggested (Watson, 1938) that, while they are feeding, aphids produce substances that inactivate such viruses. Bradley (1952) drew attention to the fact that, when *M. persicae* newly colonize a plant, they usually penetrate the leaf several times with their stylets before settling to feed continuously at one site. Both fasted and unfasted aphids do this, but Bradley concluded that fasted ones not only usually penetrate sooner, but also rarely maintain their preliminary punctures for more than 2 min., whereas unfasted ones sometimes remain with their stylets inserted for long periods. His data comparing the penetrations made by fasted and unfasted aphids show no striking differences in frequency or duration, but he suggested that they might 'account for some of the increased efficiency of vectors caused by fasting'. It is doubtful whether these differences play any major part, however, particularly the differences in duration of penetration, because most of the effects of fasting have been demonstrated by experiments in which the penetration periods of both fasted and unfasted aphids were artificially restricted to 2 min. Bradley (1952) suggested that aphids acquire such viruses as henbane mosaic only from the epidermis of infected leaves and that they cannot acquire them from deeper tissues because of the salivary sheath which surrounds the stylets when aphids feed continuously. He further suggested that fasting increases the proportion of infective aphids by increasing the proportion that feed too briefly to form a salivary sheath. As further evidence that *M. persicae* acquires potato virus *Y* from epidermal cells, Bradley (1953) reported that, of aphids that had been on infected tobacco plants for some hours, only those that had ceased feeding continuously and had recently re-inserted their stylets into the epidermis transmitted the virus when moved to healthy plants.

Bradley (1952) showed that aphids can become infective by feeding on pieces of epidermis stripped from plants infected with henbane mosaic virus. That such viruses are also readily obtained from the epidermal cells of intact leaves is evident from what is known about the rate at which aphids' stylets pass into leaves (Roberts, 1940; Bradley, 1952). This fact is particularly obvious with starved aphids, which give most transmissions after feeding for times too short for their stylets to have penetrated beyond the epidermis. At least a minute, and usually several, are needed for the stylets to reach the mesophyll, and 15 min. or more to reach the phloem, whereas fasted aphids often become infective in 20–30 sec. and most become so within 2 min.

The problem, therefore, is not whether aphids can get this type of virus from the epidermis, for this is established, but to explain the decrease in numbers of transmissions when fasted aphids prolong their feeding time on infected plants and the fact that, with short feeding periods, fasted aphids transmit more readily than unfasted ones. The idea that aphids while feeding continuously produce substances that inactivate this type of virus has the advantage of explaining both phenomena, but it is virtually untestable and has the objection that the inhibitors must be postulated to have no effect against infection by other types of insect-transmitted viruses. Bradley postulated that the viruses can be obtained only from the epidermis because the salivary sheath is impermeable to them, and only at the start of feeding are stylets free from the sheath. Again, this is an untestable postulate; even if true, however, it seems unreasonable to expect that the sheath would do more than prevent aphids acquiring virus from the mesophyll, and aphids normally seem to imbibe little of their food from this tissue (Day & Irzykiewicz, 1953; Watson & Nixon, 1953). Not only is it unlikely that aphids would hamper their feeding from the phloem by surrounding their stylets with a semi-permeable membrane, but there is every reason to think that aphids do normally get such viruses as potato leaf roll and sugar beet yellows while they are feeding from the phloem. On Bradley's theory, therefore, it becomes necessary to assume that viruses like henbane mosaic virus do not occur in the phloem, an assumption that fits ill with what is known of the manner in which they move through plants and with the fact that plants infected with henbane mosaic virus produce inclusion bodies in all tissues except the xylem (Sheffield, 1938).

The correlation between feeding on the epidermis and numbers of transmission is more plausibly explained simply by postulating that the epidermal cells contain more extractable virus than the deeper tissues. The stylets of aphids filled with sap taken from the epidermis would then be more likely to include virus than when they contain sap from other tissues. Fasting the aphids could then increase transmission by making them more likely to imbibe sap from epidermal cells. This could be simply a mechanical effect; the stylets of most fasted aphids may be empty and fill when they penetrate the epidermis, whereas the stylets of most aphids that have come directly from other leaves may already contain fluid. Alternatively, fasted aphids may be more prone to feed on the first cells they encounter. Experiments with ^{32}P (Day & Irzykiewicz, 1953; Watson & Nixon, 1953) show that even fasted aphids imbibe very little from the epidermis, almost certainly too little to be directly important in the aphids' food supply. The amount may be merely incidental to main feeding, or it may be taken more actively to test whether a new plant is an acceptable host for the aphid. Aphids that have been fasted for some time have obvious similarities to migrating aphids, and it is plausible to think they are more likely to test a leaf on which they are newly placed than aphids that are moved directly from one leaf to another.

There are, then, various reasons to assume that fasted aphids might be more prone than unfasted ones to imbibe sap initially from epidermal cells, and whether or not viruses such as henbane mosaic occur predominantly in the epidermal cells of systemically infected leaves seemed testable by the use of ultra-violet radiation.

Ultra-violet not only inactivates plant viruses, but when leaves are exposed to it there is good evidence that tissues other than the epidermis are little affected. This limitation is to be expected, as ultra-violet has little penetrating power, as was first demonstrated by Kluyver (1911). He irradiated leaves of several species for many hours and when he later sectioned them he found that, although the epidermis was entirely shrivelled, the deeper tissues were apparently unaffected. Bawden & Kleczkowski (1952) found that exposing French bean leaves to ultra-violet for a few minutes produced no immediately obvious external signs of damage, but the epidermal cells were affected because they resisted infection when inoculated with viruses to which they are normally susceptible. If placed in daylight, the irradiated leaves continued to seem unharmed and within a day they regained their initial susceptibility to infection by viruses. By contrast to their behaviour in daylight, if leaves were kept in darkness after irradiation, the epidermal cells shrivelled, but the leaves did not die and, when sectioned, the palisade and spongy parenchyma was indistinguishable from that of untreated leaves. Evidence that ultra-violet does not readily inactivate virus in cells other than the epidermis is further provided by the fact that irradiating leaves within a few hours after inoculation prevents the formation of local lesions, whereas irradiation after longer intervals does not.

These results make it reasonable to assume that, when infected leaves are irradiated, virus present in the epidermis will be inactivated but not that in the deeper tissues. We have therefore irradiated leaves infected with various viruses and tested the effect on the infectivity of sap and on the ability of aphids to transmit viruses from the leaves. The results are all compatible with the view that henbane mosaic, and other viruses that are also transmitted most readily by fasted aphids that feed on infected plants for only short periods, occur in an extractable form predominantly in the epidermis.

MATERIALS AND METHODS

Most of the experiments were done with cabbage black ring spot virus (CBRSV), using systemically infected turnip (*Brassica rapa* L., var. Early Snowball) as the source of the virus, and tobacco (*Nicotiana tabacum* L., var. White Burley), which reacts to infection with necrotic local lesions, as the test plant. Others were made with henbane mosaic virus in tobacco, and with sugar-beet mosaic and yellows viruses in sugar beet (*Beta vulgaris* L., var. Klein E).

When doing local lesion tests, the inocula were rubbed as evenly as possible over the leaves with the forefinger wet with inoculum. If the lesions were not otherwise visible, they were developed by decolorizing the inoculated leaves and staining with iodine (Holmes, 1931). The aphids (*Myzus persicae* (Sulz.)) were reared on turnip plants and handled as described by Watson (1936, 1938). They were fasted by being placed over damp filter-paper in a Petri dish with a 'Cellophane' cover, placed at 5° C. for an hour and then kept overnight at about 14° C.

The source of ultra-violet radiation was a low-pressure mercury-discharge lamp made by the Thermal Syndicate Ltd., fitted with a chromium-plated cylindrical reflector. It gives a radiation 99 % of which is of wave-length 2546 A. and at 20 cm., the distance from the lamp the leaves were placed, the intensity is 870 μW./sq.cm. When aphids were to be fed on irradiated leaves, both surfaces were irradiated so that there was no chance of their feeding on an untreated epidermis. Aphids were fed mainly on the under-surfaces of leaves. To counteract some of the damaging effects of irradiation, the leaves were floated in water with their under-surfaces uppermost and exposed to daylight for at least an hour before aphids were fed on them. Control leaves were floated in the same way. Unless otherwise stated, the time of exposure to ultra-violet was 5 min.

EXPERIMENTAL

Effects of irradiating leaves on the infectivity of sap

Table 1 shows that irradiating leaves systemically infected with CBRSV, or henbane mosaic virus, decreased the infectivity of sap to about one-fifth that of sap from unirradiated leaves. Both the upper and lower leaf surfaces were irradiated for 5 min. This time was chosen because preliminary tests, in which turnip leaves infected with CBRSV were irradiated for various periods, suggested that it gave almost the maximum inactivation. Sap from leaves irradiated for 4 min. was less infective than that from leaves irradiated for 2 min., but little more infective than sap from leaves irradiated for 8 min. Because the leaves have uneven surfaces, it is unlikely that all the epidermal cells are uniformly exposed and that all the virus in the epidermis is inactivated, but at the intensity of radiation used 5 min. seems adequate to inactivate the extractable virus in fully exposed cells. Whole leaves were detached and placed under the lamp with halves protected from the ultra-violet by a sheet of glass laid along the mid-ribs. At least six leaves were used in each test; after irradiation, they were cut along their mid-ribs, when the treated and untreated halves were bulked separately, ground with a pestle in a mortar and the sap squeezed through muslin. The fluids were diluted in water and their relative infectivities compared by the half-leaf method, each dilution being inoculated to at least eight half leaves.

In contrast to the behaviour of leaves infected with these two viruses, when tobacco leaves systemically infected with tobacco mosaic virus were treated similarly, there was little difference between the infectivity of sap from irradiated and unirradiated half leaves. This difference might be partly explained because tobacco mosaic virus is more resistant to inactivation by ultra-violet and because it occurs in much higher concentration, so that more irradiation is needed to inactivate. Even when the period of exposure was extended to 20 min., however, the infectivity of sap from irradiated halves was still more than two-thirds that of sap from unirradiated halves. This drop in infectivity is roughly proportional to the volume of the leaf provided by the two layers of epidermal cells, and it suggests that extractable tobacco mosaic virus occurs in similar concentrations in all the leaf parenchyma. The results with the two other viruses, however, suggest that they occur predominantly in the epidermal cells, which seem to contain about four-fifths of the virus set free in sap, although they amount to little more than one-fifth the total volume.

An alternative explanation of the phenomenon would be that ultra-violet not only inactivates virus in the epidermis but also produces substances that either inactivate the viruses in sap or act as inhibitors of infectivity. This seems unlikely. We have compared sap from irradiated and unirradiated leaves of healthy turnip and tobacco plants for its ability to affect the infectivity of preparations of CBRSV, henbane mosaic and tobacco mosaic viruses, and have found no differences. It seems, then, that the effects of irradiation on infectivity reflect the extent to which virus is inactivated in the epidermal cells, and the contrasting results with tobacco mosaic viruses and the aphid-transmitted ones suggest that viruses that cause mosaic-type diseases can occur in different relative concentrations in different parts of a leaf.

TABLE 1. *The decreased infectivity of sap caused by exposing virus-infected leaves to ultra-violet radiation*

		No. of lesions with diluted sap from			
		Unirradiated leaves, dilution of sap		Irradiated leaves, dilution of sap	
Infecting virus		1/25	1/125	1/5	1/25
Cabbage black ring spot	Exp. 1	9	4	10	5
	Exp. 2	15	6	14	5
		1/25	1/125	1/10	1/50
Henbane mosaic	Exp. 1	130	43	72	16
	Exp. 2	98	32	68	10

Results given as the mean number of lesions per half leaf produced by sap, at the stated dilution in water, from irradiated and unirradiated halves of systemically infected leaves.

Transmission by aphids from irradiated leaves

Table 2 shows the pooled results of several experiments made to compare the ability of fasted *M. persicae* to transmit CBRSV from irradiated and unirradiated turnip leaves to tobacco seedlings. The aphids were allowed to feed on the infected turnip leaves for 2 min., 1 or 24 hr., before they were moved to the test plants (six aphids per tobacco leaf), on which they remained over-night. The aphids penetrate the epidermis as quickly, and seem to feed as readily, on the irradiated leaves as on the controls. The only difference in behaviour we have noted is that, in some experiments, after 24 hr. fewer aphids seemed to be feeding on the irradiated than on the control leaves, and aphids on the irradiated leaves produced fewer young. The results, expressed as the total number of local lesions produced by aphids given the different infection-feeding times, show that, whereas aphids fed for 2 min. on irradiated leaves caused only a tenth as many lesions as those fed on the unirradiated leaves, those fed for 1 and 24 hr. caused as many lesions as aphids fed on unirradiated leaves.

Table 3 shows the pooled results of further experiments made to compare the relative abilities of fasted and unfasted aphids to transmit CBRSV from irradiated and unirradiated turnip leaves. As in Table 2, the only large effect of irradiation is on the number of transmissions caused by aphids given 2 min. infection feeding. A response to fasting still shows, for both fasted and unfasted aphids produced about one-tenth as many lesions as when fed on unirradiated leaves. We think it probable that this occurred because the leaf surfaces were not uniformly exposed to ultra-violet and that the virus transmitted on these occasions came from portions of the leaf shaded from the radiation. It may be that in 2 min. the stylets of some aphids do penetrate into the mesophyll of turnip leaves, but turnip leaves have such uneven surfaces that it is reasonable to expect that some of the epidermis would not meet the full irradiation. Shading of 10% of the surface would be enough to explain the transmissions and that a response to fasting is obtained with irradiated leaves.

TABLE 2. *The effect of exposing leaves to ultra-violet on the transmission of cabbage black ring spot virus by fasted* Myzus persicae

Infection-feeding times	Aphids fed on control leaves	Aphids fed on irradiated leaves
2 min.	235	20
1 hr.	14	12
24 hr.	29	21

Results are given as total of local lesions produced on fifty-six tobacco leaves.

TABLE 3. *The transmission of cabbage black ring spot virus by fasted and unfasted aphids from irradiated and unirradiated turnip leaves*

Infection-feeding times	Unirradiated leaves		Irradiated leaves	
	Fasted aphids	Unfasted aphids	Fasted aphids	Unfasted aphids
2 min.	146	35	14	4
1 hr.	8	12	11	10
24 hr.	23	26	14	23

Results are the total number of lesions produced on twenty-four tobacco leaves.

Table 4 shows pooled results of comparable experiments with *M. persicae* transmitting henbane mosaic virus from and to tobacco plants. The test seedlings were colonized with five aphids so as to get workable numbers of infections when treatments were such that only a small proportion of aphids were infective. The results are recorded as the number of plants that became infected out of those colonized. Again the largest effect of irradiation shows in the decreased number of infections caused by fasted aphids given 2 min. infection feedings, but the results do differ in two respects from those with CBRSV. First, fasted aphids given 2 min. infection feedings transmitted henbane mosaic virus no more frequently from irradiated leaves than did unfasted aphids, although they did from unirradiated leaves. Indeed the response to fasting with the unirradiated leaves was certainly much larger than our results suggest, for all the plants colonized with fasted aphids given short infection feedings became infected and most probably received more than one infective aphid. The fact that irradiating tobacco leaves abolished the response to fasting, whereas irradiating turnip leaves did not, we attribute to differences between the surfaces of the two kinds of leaves; tobacco leaves are much smoother and, when laid flat beneath the lamp, their surfaces are more uniformly exposed to the radiation, so that few epidermal cells remain unaffected to act as sources of virus. A second difference from the results with CBRSV is that, with all feeding times, and with fasted and unfasted aphids, henbane mosaic virus was transmitted less often from irradiated than from unirradiated leaves. The fewer transmissions obtained with 1 and 24 hr. infection feedings suggests, at first sight, that irradiation may have inactivated virus in cells beneath the epidermis. The result is more probably explained, however, by Bradley's (1953) observation that *M. persicae* do not all feed continuously when colonizing tobacco leaves, and that those that temporarily cease feeding and newly re-insert their stylets into the epidermis are the most likely to transmit. Aphids that do this on unirradiated leaves could acquire virus, whereas they could not on irradiated ones.

TABLE 4. *The transmission of henbane mosaic virus by fasted and unfasted aphids from irradiated and unirradiated tobacco leaves*

Infection-feeding times	Unirradiated leaves		Irradiated leaves	
	Fasted aphids	Unfasted aphids	Fasted aphids	Unfasted aphids
2 min.	20	9	1	1
1 hr.	5	7	1	1
24 hr.	7	8	3	3

Results are number of tobacco seedlings that became systemically infected out of twenty, each colonized by four aphids.

Table 5 shows the results of additional experiments to compare the behaviour of CBRSV and henbane mosaic virus when aphids were given infection-feeding times of 1 and 24 hr. on irradiated and unirradiated leaves. Again, CBRSV was transmitted almost equally readily from both lots of leaves, whereas henbane mosaic virus was transmitted much less frequently from the irradiated than from the unirradiated leaves. The difference is particularly noticeable with the 1 hr. infection-feeding time. We attribute this difference, not to any differential effect of ultraviolet on the individual viruses in the two host plants, but to the behaviour of the aphids in the conditions of our experiments. Not only is turnip a normal host plant for *M. persicae*, whereas tobacco is not, but the aphids were feeding on turnips before being used in the transmission tests. The transfer from one turnip plant to another is a minor change, comparable only with moving position on the same leaf, but transfer to tobacco could be expected to alter the aphid's behaviour considerably. Some time would be needed for the aphid to accept the new host, and for the first hour it is unlikely that many become so accustomed to it that they will feed continuously from the phloem. Most transmissions that occur during this time, therefore, may be caused by aphids that are tentatively probing the epidermis. Transfer to a new host possibly produces responses equivalent to those produced by fasting, and it seems that more detailed observations than are usually made of the feeding behaviour of aphids when transferred from one species to another might be valuable in helping to explain the results of virus-transmission experiments.

TABLE 5. *The transmission of cabbage black ring spot and henbane mosaic viruses after long infection feeding on irradiated and unirradiated leaves*

Infection-feeding times	Cabbage black ring spot		Henbane mosaic	
	Control leaves	Irradiated leaves	Control leaves	Irradiated leaves
1 hr.	31	25	33	3
24 hr.	63	58	32	15

Results with CBRSV are the total number of local lesions on forty-eight tobacco leaves, each colonized with six aphids, and with henbane mosaic virus they are the number of plants that became systemically infected out of forty-eight, each colonized with four aphids.

Although all our results suggest that both CBRSV and henbane mosaic virus are most readily obtained by aphids from the epidermis of systemically infected leaves, they do not support Bradley's (1952, 1953) suggestion that this is the only tissue from which they can be obtained. The virus transmitted by aphids that were on irradiated turnip leaves for 24 hr. almost certainly came mostly from deeper cells. When fasted or unfasted aphids were fed for 2 min. on irradiated leaves at different times up to 3 days after the exposure to ultra-violet, they transmitted no more frequently than at the start of the experiment. As the short infection feedings always produced fewer transmissions than long ones, it seems unreasonable to postulate that those obtained with the long ones occurred because the aphids temporarily ceased feeding on the phloem and fed momentarily on the epidermis. The frequency with which this normally occurs is perhaps indicated by the difference in numbers of transmissions obtained from irradiated and unirradiated leaves by aphids fed for 24 hr. The difference suggests that rather more than half the transmissions of henbane mosaic virus by aphids given long infection feedings on tobacco occur because of virus acquired from the epidermis. Transmissions of CBRSV in this manner are apparently rare, and it seems that *M. persicae* when colonizing turnip for long periods feed continuously on deep-seated tissues and derive the virus from them.

Some experiments with sugar-beet leaves infected with beet mosaic and beet yellows viruses provided further evidence that ultra-violet probably only inactivates virus in epidermal cells, and that different viruses are differently distributed in leaves. Sugar-beet mosaic virus behaves like henbane mosaic virus in insect-transmission tests, whereas sugar-beet yellows does not; aphids given short infection feedings do not become infective, and yellows virus is increasingly likely to be transmitted the longer aphids feed on infected plants. Of thirty fasted aphids fed for

2 min. on infected leaves, eight transmitted mosaic virus, whereas out of thirty fed on irradiated leaves only one transmitted. Too few transmissions of mosaic virus were obtained with aphids given long infection feedings for any conclusions to be drawn from the results. That virus in the phloem is largely unaffected was suggested by results with sugar-beet yellows virus. Of fifty-four aphids given infection feedings of 4 hr., eighteen transmitted from unirradiated leaves and fourteen from irradiated leaves.

Discussion

Aphids that have fed on plants infected with such viruses as henbane mosaic and CBRSV soon lose their ability to infect plants, and lose it more rapidly when they are feeding than when they are not. The process of acquiring henbane mosaic virus (Bradley, 1952) and CBRSV (Hamlyn, 1953) from infected leaves and transmitting them to healthy plants can be completed by aphids in less than 1 min. These facts seem to preclude any idea that transmitted virus is taken into the aphid's stomach, circulates through its blood stream and is then secreted from the salivary glands, as seems to happen with other kinds of viruses whose vectors remain infective for long periods. They make almost inescapable the conclusion that transmitted virus is only that present in the fluid contained in aphids' stylets when they are withdrawn from infected plants. In conjunction with this conclusion, our results satisfactorily explain some of the previously puzzling phenomena disclosed by experiments on the transmission of such viruses by aphids. The factor mainly responsible seems to be the unequal distribution of readily extractable virus in different tissues of systemically infected leaves and its relatively high concentration in epidermal cells.

The reason why fasted aphids transmit more frequently than unfasted ones when both are given short infection feedings remains uncertain, but it can safely be assumed that fasted aphids do in these circumstances imbibe sap that is relatively rich in virus. As their stay on infected leaves is prolonged, their stylets penetrate into deeper tissues and the fasted aphids become less likely to transmit, not because they have ceased to imbibe virus, but because the fluid previously in their stylets has now passed into their stomachs and has been replaced by one with a smaller virus content.

Not all viruses whose vectors remain infective for short periods are transmitted optimally by fasted aphids that feed for short periods on infected plants. Of two that infect lettuce, for example, lettuce mosaic virus is, and dandelion yellow mosaic virus is not (Kassanis, 1947). We have made no experiments with these two viruses, but a plausible explanation for the differences is that, whereas lettuce mosaic resembles CBRSV and henbane mosaic virus and occurs predominantly in the epidermis, dandelion mosaic virus does not.

Although a different distribution of viruses in different cells of infected leaves conveniently explains some of the odd phenomena encountered in studying the transmission of viruses by aphids, others still remain without explanation. Prime among these are the specific transmission of some viruses by one species of aphid and not by a second, although the second may seem to feed similarly and be a vector for other viruses that infect the same host plants. Examples are *Myzus ornatus* Laing, which transmits cauliflower mosaic virus and dandelion mosaic virus but not CBRSV or lettuce mosaic, and *M. persicae* which transmits lettuce mosaic but not dandelion mosaic virus (Kvíčala, 1945; Kassanis, 1947). Equally inexplicable, if CBRSV and henbane mosaic virus are carried in sap recently imbibed by aphids' stylets, is how any aphid that feeds on any tissues of leaves systemically infected with tobacco mosaic virus manages not to infect healthy plants on which it subsequently feeds.

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SAP-TRANSMISSIBLE MOSAIC DISEASES OF SOLANACEOUS CROPS IN TRINIDAD

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(With Plate 10)

Four sap-transmissible viruses were isolated from cultivated Solanaceae in Trinidad: (1) tobacco mosaic virus, from tobacco, tomato and sweet pepper; (2) cucumber mosaic virus, from tobacco and petunia; (3) 'pepper vein-banding virus', probably related to pepper mosaic viruses in Puerto Rico and Brazil, from peppers and tobacco; (4) 'egg-plant mosaic virus', possibly related to the tobacco ring-spot virus, from egg-plant and tomato. Pepper vein-banding virus causes leaf-crinkling and vein-banding in *Physalis floridana*, petunia, various *Nicotiana* spp. and most peppers; the Large Bell Hot pepper is killed; tomato and egg-plant are immune. Egg-plant mosaic virus produces mosaic, ring-spotting, or both, on different solanaceous species. It also gives local and systemic ring-spotting on *Chenopodium hybridum* and necrotic local lesions on the primary leaves of cowpea (var. Black-eye); cucumber is a symptomless carrier. Only cucumber mosaic virus was found naturally infecting non-solanaceous hosts, cucumber and certain common wild plants.

The thermal inactivation point of pepper vein-banding virus is 62° C., its dilution end-point 2×10^{-5} and its longevity *in vitro* 6 days at 25–30° C.; corresponding values for egg-plant mosaic virus are 78° C., 10^{-6} and over 3 weeks. *Aphis gossypii* transmits cucumber mosaic and pepper vein-banding, but not egg-plant mosaic, of which *Epitrix* sp. is an occasional vector. Tobacco mosaic, as elsewhere, probably has no regular insect vectors in Trinidad.

Introduction

Since Dale (1943) reported tobacco mosaic virus in Trinidad, in tobacco (*Nicotiana tabacum* L.), pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.), it has seldom been seen, but strains of cucumber mosaic have been common in tobacco. This virus was identified immunologically, using Price's (1935) indicator strain. It has also been isolated from mottled *Petunia hybrida* Vilm., cucumber (*Cucumis sativus* L.), *Alternanthera ficoidea* (L.) R.Br., *Commelina diffusa* Burm. f. and *C. elegans* H.B.K. The last three are very common weeds, and strains of the same virus are probably responsible for some of the other mosaics of wild plants in the island.

Two other sap-transmissible viruses of solanaceous crops have been recognized since 1944. One is common in peppers (*Capsicum annuum* and *C. frutescens* L.) and occurs also in tobacco; it is probable that symptoms on *C. annuum* ascribed to tobacco mosaic (Dale, 1943) were due to a mixture of it with this pepper virus.

A second, widespread in egg-plants (*Solanum melongena* L.), also occasionally causes mosaic in adjacent tomatoes. Brief notes on these two viruses, which he called 'pepper mosaic' and 'egg-plant mosaic' respectively, were published by Ferguson (1951). The name 'pepper vein-banding virus' is preferred for the former, because 'pepper mosaic' has been used elsewhere for the disease due to tobacco mosaic virus.

HOST REACTIONS

Pepper vein-banding virus

This is readily sap-transmissible by rubbing, using carborundum powder. On most pepper varieties slight vein-clearing of the expanding leaves developed in 7–10 days, followed by mottling. Ultimately the usual leaf pattern was an irregular and discontinuous dark green vein-banding, often bordered by chlorotic areas which merged into darker interveinal tissue (Pl. 10, fig. 1*a*). This mottling was usually accompanied by crinkling and reduction in size of the lamina (Pl. 10, fig. 1*b*). The intensity of field symptoms depends on the variety, and on growth conditions, but different strains of the virus may exist. Infected plants of most large-fruited sweet pepper varieties (*Capsicum annuum* var. *grossum*), such as California Wonder or Fordhook, are stunted, and give reduced yields of smaller and often misshapen fruits. Puerto Rico Wonder develops milder leaf symptoms and is harder to infect. This variety was produced in Puerto Rico by incorporating into California Wonder the resistance shown by Cuaresmeño, a Mexican pungent variety, to a pepper mosaic there (Roque & Adsuar, 1941; Riollano, Adsuar & Rodriguez, 1948). Certain local red- and yellow-fruited hot peppers (*C. annuum* var. *minimum*) showed resistance to infection with the vein-banding virus in Trinidad. When infected, they gave mild leaf symptoms and were not stunted. Perennial small-fruited bird peppers and cherry peppers (*C. frutescens*), common around gardens and abandoned cultivations, often show clear leaf symptoms, but no other obvious damage. Young plants of the Large Bell Hot variety of *C. annuum* gave dark brown vein-necrosis of the developing leaves 4–8 days after inoculation (Pl. 10, fig. 1*c*), followed rapidly by necrotic streaking of the stem and abscission of all but the oldest leaves, culminating in death of the plant. Dark brown necrotic local lesions occasionally appeared a day or two before the systemic necrosis. Older plants turned chlorotic, developed necrosis of the active shoots and shed their fruits, but often survived in a moribund condition for some time. Some hybrids between Large Bell Hot and other varieties showed an additional type of response. They were stunted and produced little or no fruit; their leaves, which were small, mottled and curled downwards, showed varying degrees of vein-necrosis. The genetics of these responses will be described elsewhere.

Infected field tobacco shows dark-green blotches, sometimes ill-defined, along the veins. Young White Burley plants inoculated in the glasshouse developed vein-clearing, followed by discontinuous dark green vein-banding, sometimes bordered by chlorotic areas which merged into the normal leaf colour. Almost identical reactions occurred in *Nicotiana tabacum* var. *macrophylla* (an ornamental tobacco), *N. chinensis* Fisch. and *N. sylvestris* Speg. & Comes. The lesions on *N. alata* var. *grandiflora* Comes, *N. glutinosa* L., *N. rustica* L. and *N. sanderae* Hort. differed only in degree, being inconsiderable and restricted to small areas, often towards the apex, on a few leaves. Ferguson (1951) either failed to infect *N. glutinosa, N. rustica* and *N. sylvestris* or overlooked their reactions. Petunia and *Physalis floridana* Rydb. showed more obvious symptoms than most *Nicotiana* spp., but of the same general type. The following were not susceptible to the virus: tomato, egg-plant, celery (*Apium graveolens* L.), cucumber, pumpkin (*Cucurbita pepo* L.), *Chenopodium hybridum* L., *Phaseolus vulgaris* L., cowpea (*Vigna unguiculata* (L.) Walp.) and *Zinnia elegans* Jacq.

Egg-plant mosaic virus

This is more readily sap-transmissible than pepper vein-banding or cucumber mosaic, but less so than tobacco mosaic. Yellowish local lesions, up to 3 mm. across, often appeared on inoculated egg-plants after 2–4 days. Vein-clearing of the expanding leaves, including the inoculated one if small, appeared in 4–8 days, followed by mottling which finally took the form of broad irregular dark green vein-banding. Variation in symptom intensity occurs, especially in the field. At times, the leaves show only faint mottling; at others, the interveinal areas, of lower ones in particular, become quite yellow (Pl. 10, fig. 2). The infection of young plants causes a temporary growth check, but there is no evidence of reduction in final size or yield.

The inoculation of tomato or *Lycopersicon pimpinellifolium* Mill. produced chlorotic local lesions about 6 mm. in diameter, with indefinite margins. Vein-clearing occurred a few days later, followed by light and dark green mottling and slight leaf-crinkling. This virus stunted tomatoes more than tobacco mosaic or a strain of cucumber mosaic isolated from tobacco. The few tomatoes found naturally infected harboured also a common tomato virus disease, transmissible by grafting but not by rubbing, which Ferguson (1951) called 'twisted leaf'. The double infection severely stunted the plants. Their leaves were curled and showed whitish yellow interveinal flecks. In a field trial during the dry season, when tomatoes are most commonly grown, egg-plant mosaic alone, and the two viruses jointly, reduced yield by 25 and 46% respectively.

The Arran Pilot potato (*Solanum tuberosum* L.) (a crop which cannot be successfully grown in Trinidad) is susceptible to egg-plant mosaic. It reacted (in a single trial) by developing a few circular chlorotic local lesions, which later became necrotic. The next few leaves to expand showed similar but more ring-spots, some of which also turned necrotic. Subsequent leaves became faintly mottled, but it is not certain that this was due to egg-plant mosaic. Inoculated leaves of pepper (vars. Fordhook, Puerto Rico Wonder and Large Bell Hot) developed rather irregular yellowish local lesions, usually about 3 mm., but sometimes up to 8 mm., in diameter.

According to Ferguson (1951), a few similar spots may appear on the next leaves to expand, but this does not seem to be usual; nor has it been possible to substantiate his claim that virus is recoverable from leaves other than the ones inoculated. In petunia, chlorotic local lesions were followed successively by yellowish vein-banding, and coarse light and dark green leaf-mottling, the latter ultimately tending towards dark green vein-banding. Yellow local lesions, 1–2 mm. across, and fairly mild coarse mottling developed on *Physalis floridana.*

Nicotiana glutinosa gave circular yellowish local lesions of 1–3 mm. diameter, which usually soon turned whitish and necrotic. They were often followed by chlorotic ring-spots, becoming at times necrotic or partially so, on one or more leaves above the inoculated ones (Pl. 10, fig. 3*a*). Later leaves usually developed bright yellow vein-banding a few days after (Pl. 10, fig. 3*b*), which turned into a light and dark green mottling, tending towards irregular dark green vein-banding (Pl. 10, fig. 3*c*). On some leaves, ring-spotting or chlorotic vein-banding did not become general. The plants became stunted, particularly if inoculated young. When inoculum was obtained from long-infected egg-plants, particularly old ones in the field, few local lesions developed, and sometimes only ring-spots or no systemic symptoms at all; on the other hand, juice from recently infected egg-plants usually induced many local lesions followed by yellow vein-banding. Such behaviour suggested that the symptoms described on *N. glutinosa* might be due to two distinct viruses, one producing ring-spotting and the other vein-banding and mottling. This, however, is unlikely, for not only could the full set of symptoms be obtained on *N. glutinosa* by inoculating from plants of the same species showing only ring-spotting, but heating or diluting infective juice, or passage through various unrelated hosts, produced no permanent change of symptoms. Under the prevailing glasshouse conditions, *N. glutinosa* plants in small pots often flowered very early, which prevented full symptom expression; when plants showing only local lesions were transferred to larger pots and their inflorescences were removed, systemic symptoms appeared on some of the side shoots.

Young White Burley tobacco plants developed circular chlorotic local lesions, 1–3 mm. across, sometimes with necrotic margins. No further symptoms appeared and the virus was not recovered from uninoculated leaves. Older or poorly growing plants gave no lesions, which may explain the failure of Ferguson (1951) to infect this host. He also listed *N. alata* var. *grandiflora*, *N. chinensis*, *N. rustica*, *N. sanderae* and *N. sylvestris* as insusceptible, and in limited trials I have also failed to infect them.

Chenopodium hybridum gave necrotic local lesions about 1 mm. across, and systemic chlorotic ring-spots, sometimes turning necrotic from their centres. The systemic spots were few on young leaves, but became numerous as these matured, and sometimes coalesced to give yellowish mottling. Some variation in the type of systemic symptoms occurred in this host, as in *Nicotiana glutinosa.* The primary leaves of young Black-eye cowpeas developed reddish brown necrotic spots, 1–2 mm. across.

The virus was recovered from the inoculated leaves of cucumbers, which remained symptomless, but celery, pumpkin and zinnia were not infected. Ferguson (1951) listed cucumber as insusceptible.

PROPERTIES

The inactivation temperature, dilution end-point and longevity *in vitro* of the four viruses were determined in the usual way. For tobacco mosaic and cucumber mosaic the results agreed with those of previous workers. The Large Bell Hot pepper was a satisfactory test host for pepper vein-banding, which was obtained from recently infected Fordhook plants for most of the trials. In the absence of a known reliable local lesion host, young egg-plants were used as test plants for egg-plant mosaic. Young, recently infected egg-plants, petunias, *Physalis floridana* and *Nicotiana glutinosa* were used as source plants in different trials, which all gave similar results. The chlorotic local lesions, usually discernible on the test plants, decreased in number as the inactivation points for the three treatments were approached. The results obtained with both viruses are compared in Table 1. Egg-plant mosaic does not seem to have a high temperature coefficient of thermal inactivation, and pepper vein-banding may be similar in this respect. Ferguson (1951) gave 65° C. as the heat inactivation temperature of the former, and a lower dilution tolerance for both. These results, particularly the first, suggest unsatisfactory techniques. He obtained infections with juice aged at 15° C. *in vitro*, up to 21 days for egg-plant mosaic and 16 days for pepper vein-banding. Retention of infectivity for shorter periods at 25–30° C. is to be expected.

TABLE 1. *Properties of egg-plant mosaic and pepper vein-banding viruses*

Heat inactivation (10 min. heating)

Egg-plant mosaic		Pepper vein-banding	
Temp. (° C.)	Plants infected*	Temp. (° C.)	Plants infected*
30	40	30	40
66	32	52	38
72	18	58	22
74	7	60	12
76	6	61	6
78	0	62	0

Dilution end-point

Egg-plant mosaic	Pepper vein-banding
10^{-6}	2×10^{-5}

Longevity *in vitro* (in days, at 25–30° C.)

Egg-plant mosaic	Pepper vein-banding
Over 21	6

* In five trials with eight plants in each.

TRANSMISSION

Pepper vein-banding and egg-plant mosaic are much more common in Trinidad than tobacco mosaic virus. Although neither is seed-borne, they usually appear early, and spread rapidly. *Aphis gossypii* Glov., long known as a vector of cucumber mosaic virus in U.S.A. (Doolittle, 1920), transmits both this and pepper vein-banding in Trinidad, where it is the commonest aphid and probably the chief field vector of the two viruses. *Myzus persicae* (Sulz.), widely reported as a vector of cucumber mosaic virus, and found to transmit the pepper mosaic of Puerto Rico (Roque & Adsuar, 1941), has been collected in Trinidad, but is rare. It is one of the four aphid species (two undetermined) that are known to transmit a pepper mosaic in Brazil (Costa & Alves, 1950). In a preliminary trial, both cucumber mosaic and pepper vein-banding viruses were transmitted more readily by fasted aphids fed on infected plants for 5 min. only, than by those given longer infection feeding. Cucumber mosaic virus was already known to behave in this manner, characteristic of certain 'non-persistent' viruses (Watson & Roberts, 1939; Bhargava, 1951). Pepper vein-banding probably behaves similarly. Flea-beetles (*Epitrix* sp.) can transmit egg-plant mosaic. Although they do not transmit very readily and soon lose their infectivity, their abundance and activity may be responsible for the high incidence of the disease. *Aphis gossypii* is also common on egg-plants, but did not transmit in repeated transmission tests. No other insects have yet been tested.

RELATIONSHIPS OF PEPPER VEIN-BANDING AND EGG-PLANT MOSAIC VIRUSES

The infection of *Nicotiana glutinosa* by pepper vein-banding did not prevent the appearance of local lesions and systemic symptoms following inoculation with egg-plant mosaic, and the two were subsequently separated by passing the mixture through pepper and egg-plant. Neither virus protected *N. glutinosa* against tobacco mosaic or cucumber mosaic viruses. Similarly, *Chenopodium hybridum* infected with egg-plant mosaic virus still developed characteristic local lesions when inoculated with cucumber mosaic virus (Roberts, Wilkinson & Ross, 1951), and Large Bell Hot peppers infected by either tobacco mosaic or cucumber mosaic viruses still succumbed to inoculation with pepper vein-banding virus. Although the protection afforded by one strain against infection by others of the same virus is not always complete (Fulton, 1951; Matthews, 1949), the above results, coupled with differences in host ranges and properties, clearly suggest that the four viruses are unrelated.

Peppers are susceptible to various sap-transmissible viruses in addition to tobacco mosaic and cucumber mosaic. According to Roque & Adsuar (1941), the pepper mosaic of Puerto Rico, unlike pepper vein-banding, seemed not to infect *Nicotiana sylvestris*; occasional symptoms on egg-plant (Puerto Rican Beauty) and tomato (Marglobe) were attributed to it, though the virus could not be recovered by inoculating to Large Bell Hot peppers. The Puerto Rican virus also seemed less resistant than pepper vein-banding virus to dilution (in particular), heating, and ageing *in vitro*. However, the similarity of symptoms produced by the two viruses on pepper varieties, including Large Bell Hot (which reacts so characteristically to both), is unlikely to be fortuitous. Some of the differences may be more apparent than real, for Roque & Adsuar (1941) make no mention of having used carborundum powder in their rather unusual inoculation technique. The Puerto Rican virus, like pepper vein-banding, was shown to be immunologically unrelated to cucumber mosaic and tobacco mosaic, and to potato viruses *X* and *Y* in addition. The last two, as well as potato aucuba mosaic, tobacco etch (Holmes, 1939) and strains of lucerne (alfalfa) mosaic (Kovačevski, 1942; Berkeley, 1947), differ in various respects from pepper vein-banding. Roque & Adsuar (1941) pointed out the similarity of symptoms produced on tobacco by their pepper mosaic and potato virus *A*. The Trinidad virus elicits a similar mild reaction, but seems to have little else in common with virus *A*. Costa & Alves (1950) described a common pepper mosaic in Brazil, which gives symptoms closely resembling those of pepper vein-banding, and likewise milder on Puerto Rico Wonder than on other varieties. They pointed out the similarity of this virus to the Puerto Rican one but classified it with the potato virus *Y* group.

There are several reports of the infection of egg-plants by sap-transmissible viruses, but cucumber mosaic and tobacco mosaic are most commonly concerned. Of the remainder, potato yellow dwarf (Holmes, 1939; Black, 1951) and strains of lucerne mosaic (Berkeley, 1947) show no obvious relationship to egg-plant mosaic. The absence of streak symptoms in tomatoes infected simultaneously with this and tobacco mosaic virus suggests lack of affinity with potato virus *X*; though the Trinidad virus does show some features in common with virus *X*, not all strains of which may give the synergistic response. Egg-plant yellows in Texas is considered due to a strain of tobacco ring-spot (Valleau, 1951). Though egg-plant mosaic differs in several respects, its behaviour in *Nicotiana glutinosa* is similar to that of ring-spot in tobacco, and there are host range similarities. The two viruses may be related.

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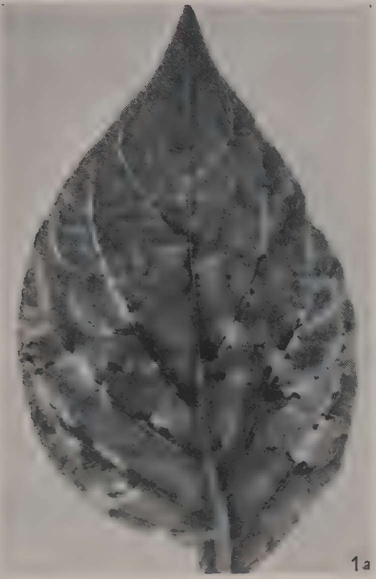
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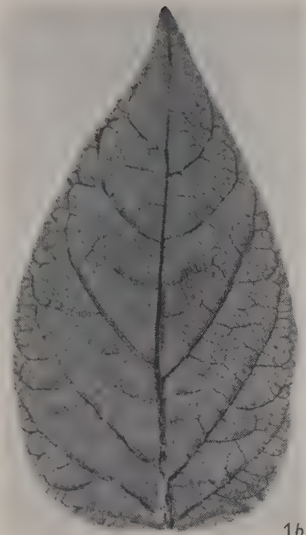
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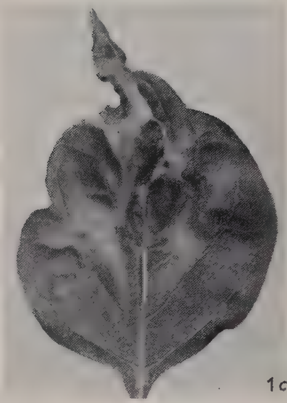
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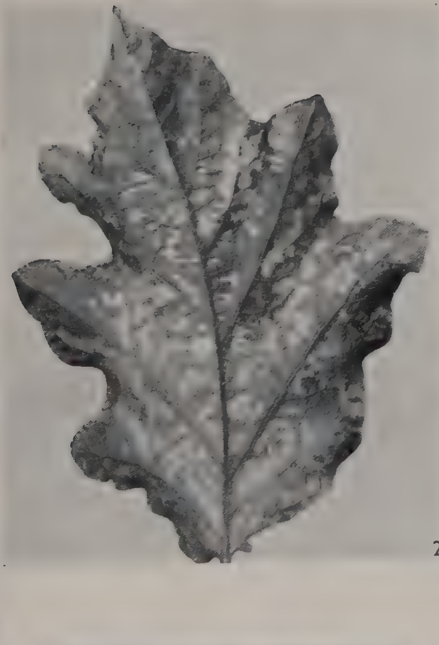
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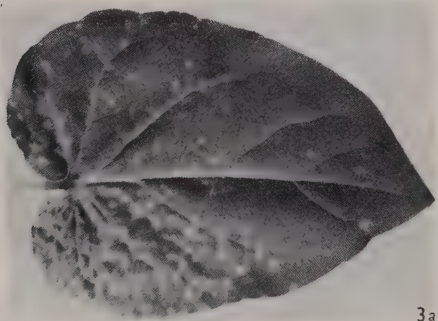
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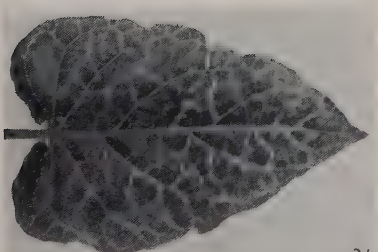
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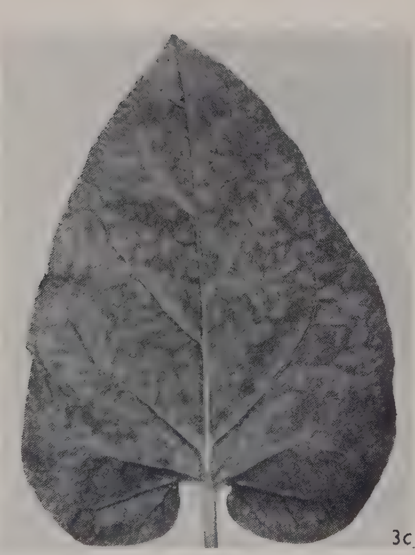
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EXPLANATION OF PLATE 10

Fig. 1. Leaves of peppers infected by pepper vein-banding virus: *a*, discontinuous dark green vein-banding in Fordhook; *b*, mottling and crinkling in the same variety; *c*, vein-necrosis in Large Bell Hot.

Fig. 2. Leaf of egg-plant infected by egg-plant mosaic virus, showing rather more intense symptoms than usual.

Fig. 3. Leaves of *N. glutinosa* showing successive systemic symptoms due to egg-plant mosaic virus: *a*, chlorotic ring-spots, some becoming partially necrotic; *b*, chlorotic vein-banding; *c*, dark green vein-banding.

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STUDIES IN THE MODE OF ACTION OF INSECTICIDES

II. INHIBITION OF THE ACETYLESTERASES OF THE LOCUST NERVE CORD BY SOME ORGANIC PHOSPHORIC ESTERS

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Homogenates of the thoracic nervous system of *Locusta migratoria migratoriodes* are able to hydrolyse acetylcholine (ACh) and *o*-nitrophenylacetate (NPA), and this hydrolysis can be inhibited by tetraethylpyrophosphate (TEPP) at approximately the same molar concentration for both substrates. It is possible that one acetylesterase is responsible for the breakdown of the two substances, and there is no reason to assume the existence of a specific acetylcholinesterase. In normal horse serum, on the other hand, the pseudocholinesterase is quite distinct from the enzyme responsible for the breakdown of NPA.

In an attempt to correlate the inhibition of the locust nerve cord acetylesterase with toxic activity to insects and mice, four chlorinated diethyl-phenylphosphates were tested as contact poisons against a number of insects and by injection against locusts and mice, and also as *in vitro* inhibitors of locust nerve cord acetylesterase and horse-serum pseudocholinesterase. The chemicals were the 2-chloro-, 4-chloro, 2:4-dichloro- and 2:4:5-trichloro- analogues of diethyl-phenylphosphate.

Good correlation exists between their *in vitro* activity against the nerve-cord acetylesterase and their contact activity to aphids, but not between the former and injection toxicity to locusts. No correlation could be established between the inhibition of horse-serum cholinesterase and injection toxicity to mice. It is thought likely that the inhibition of nerve-cord acetylesterase is of greater importance in aphids than in other insects, where the toxic action of the phosphoric esters is at least partly concerned with other vital processes, and that a detoxication mechanism in the mammal breaks down some of the phosphoric esters, but not others.

Introduction

In a previous paper (Hopf, 1952) some evidence was given throwing doubt on the validity of the widely held theory that the action of phosphoric insecticides was to inhibit acetylcholinesterase in the insect nervous system. Injections into the locust of various cholinesters and of prostigmine produced no symptoms. Eserine was considerably less toxic than it is to mammals. Adrenaline and tubocurarine had no effect whatever, and the toxic action of tetraethylpyrophosphate (TEPP) could not be influenced by injection of atropine, tubocurarine or acetylcholine (ACh).

It was concluded that there was strong evidence that the neuro/motor mechanism of transmission in the insect is not identical with that in the mammal. A tentative suggestion was made that the action of TEPP (and other substances) was likely to be a general esterase which happened to split acetylcholine but might fulfil a quite different function in the insect nerve tissue.

While these results were being prepared for publication, Lord & Potter (1951) reported that they had found an enzyme in extracts of *Tenebrio molitor* which split *o*-nitrophenylacetate (NPA) and ethyl butyrate but not acetylcholine, and which could be inhibited by TEPP, the ID_{50} being in the order of 10^{-7} M. Clearly a study of this enzyme and its behaviour towards insecticides and mammalian cholinesterase inhibitors was desirable.

It was decided to do this work on a comparative basis, testing the enzyme preparation from the insect—a *brei* of the locust nerve cord—and a mammalian source of cholinesterase against the same chemicals. Normal horse serum was chosen for this purpose. The cholinesterase of serum is, of course, not the specific acetylcholinesterase occurring in mammalian synaptic transmission. It is a so-called 'pseudocholinesterase' quite distinct from the former. Nevertheless, it was used, at least in the early stages of the work, as an indication of mammalian susceptibility. The cholinesterase of blood serum is constantly used in practice for tests on the hazards of phosphoric insecticides (e.g. Metcalf, 1951). An additional advantage was that this serum contains an enzyme readily splitting *o*-nitrophenylacetate.

The first part of the present work is concerned with inhibition by TEPP only, and the results of these series are given in detail in Tables 1–4 to demonstrate the technique, the type of graph obtained and the experimental error.

At the time of these experiments, work at these laboratories had indicated that certain dialkyl-*n*-halophenylphosphates combined fair insecticidal efficacy with low mammalian toxicity. Consequently, the following were chosen for physiological work from a number of chemicals prepared in pure form by the I.C.I. Widnes Laboratory:

diethyl-4-chlorophenylphosphate (4-Cl),
diethyl-2-chlorophenylphosphate (2-Cl),
diethyl-2:4-dichlorophenylphosphate (2:4-Cl),
diethyl-2:4:5-trichlorophenylphosphate (2:4:5-Cl).

These will be referred to by the abbreviations shown.

Results with these chemicals are summarized in Table 5. The following comparisons were made:

(1) Effect of spraying the test organisms and their food with aqueous dispersions of 0·3 and 0·06% active principle.

(2) Contact activity against aphids.

(3) Injection toxicity to locusts.

(4) *In vitro* inhibition of the acetylesterases occurring in the nerve cord of locusts.

(5) Acetylesterase activity of the nerve cord of locusts previously injected with these substances.

(6) Intra-peritoneal injection into mice at 50 mg./kg.

(7) *In vitro* inhibition of the cholinesterase occurring in normal horse serum.

These results are compared in Table 5 with those obtained with TEPP.

A comparison with diethyl-4-nitrophenylphosphate (E 600) would have been preferable but this substance was not available in known purity.

Table 1. *Inhibition of the enzymic decomposition of* o-*nitrophenylacetate by locust nerve cord homogenates*

Exp.	Concn. of TEPP (M × 10^{-8})	Enzymic production of nitrophenol (M × 10^{-3})	% substrate decomposed	% inhibition	Mean % inhibition
A	—	0·88	44	—	—
B		1·42	71	—	—
C		1·09	59	—	—
D		1·09	59	—	—
A	10	0·02	5	89	89
B	8	0·26	13	82	80
C		0·34	17	77	
B	4	0·74	37	48	46
C		0·58	29	44	
B	2	0·86	43	39	33
C		0·76	38	27	
A	1	0·66	30	32	29
D		0·88	44	25	
D	0·5	1·02	52	12	12
D	0·25	1·03	53	10	10
A	0·1	0·80	40	9	9

Duration, 60 min. Approximate $ID_{50} = 2{\cdot}8 \times 10^{-8}$ M TEPP.

Table 2. *Inhibition of the enzymic decomposition of acetylcholine chloride by locust nerve cord homogenates*

Exp.	Concn. of TEPP (M × 10^{-8})	Acetylcholine left intact (M × 10^{-3})	% substrate decomposed	% inhibition	Mean % inhibition
A	—	1·0	50	—	—
B		0·86	57	—	—
C		0·82	59	—	—
D		0·96	52	—	—
A	10	1·80	10	80	80
B	8	1·82	9	84	68
C		1·42	29	51	
B	4	1·14	43	25	31
C		1·26	37	37	
B	2	1·18	41	28	17
C		0·88	56	5	
A	1	1·20	40	20	18
D		1·12	44	15	
D	0·5	1·02	49	5	—
D	0·25	1·04	48	8	4
A	0·1	0·98	51	0	3

Duration, 120 min. Approximate $ID_{50} = 4{\cdot}6 \times 10^{-8}$ M TEPP.

TABLE 3. *Inhibition of the enzymic decomposition of* o-*nitrophenylacetate by normal horse serum*

Exp.	Concn. of TEPP (M)	Enzymic production of nitrophenol ($M \times 10^{-3}$)	% substrate decomposed	% inhibition	Mean % inhibition
A	—	1·44	72	—	—
B		1·54	77	—	—
C		1·48	74	—	—
D		1·62	81	—	—
B	2×10^{-4}	0·62	31	59	61
C		0·56	28	62	
A	1×10^{-4}	0·66	33	54	54
B		0·68	34	56	
C		0·70	35	53	
B	5×10^{-5}	0·78	39	49	51
C		0·72	36	52	
D	2×10^{-5}	1·14	57	28	28
A	1×10^{-5}	0·98	49	32	32
D	2×10^{-6}	1·70	85	+5	More active than control
A	1×10^{-6}	1·50	75	+4	
D	2×10^{-7}	1·68	84	+4	

Duration, 60 min. Approximate $ID_{50} = 5 \times 10^{-5}$ M TEPP.

TABLE 4. *Inhibition of the enzymic decomposition of acetylcholine chloride by normal horse serum*

Exp.	Concn. of TEPP (M)	Acetylcholine left intact ($M \times 10^{-3}$)	% substrate decomposed	% inhibition	Mean % inhibition
A	—	0·56	72	—	—
B		0·94	53	—	—
C		0·52	74	—	—
A	1×10^{-6}	2·0	0	100	100
A	1×10^{-7}	1·86	7	90	90
C	5×10^{-8}	1·80	10	86	86
A	1×10^{-8}	1·92	4	94	85
B		1·74	13	75	
C		1·78	11	85	
C	2×10^{-9}	1·62	19	74	74
A	1×10^{-9}	1·62	19	74	61
B		1·44	28	48	
C	4×10^{-10}	1·08	46	52	52
A	1×10^{-10}	1·58	21	71	47
B		1·18	41	23	
C	8×10^{-11}	1·00	50	32	32
C	$1·6 \times 10^{-11}$	0·78	61	18	18
B	1×10^{-11}	1·24	38	28	28
C	$3·2 \times 10^{-12}$	0·82	59	20	20

Duration, 90 min. Approximate $ID_{50} = 4 \times 10^{-10}$ M TEPP.

TABLE 5. *Summary of results obtained with diethyl-n-chlorophenylphosphate*

	Insects														Mammals	
	General contact and stomach poison test*									Acetylesterases of the nerve cord						
								Contact toxicity to *Macrosiphum pisi* (*Aphis*) MLD in % concn.	Injection toxicity to *Locusta* MLD in g./locust	*In vitro* ID$_{50}$ in molar concn. with substrate		*In vivo* Inhibition 4 hr. after injection compared with control (%)			I.P. inject. 50 mg./kg. of mice: number killed out of 10	Horse serum cholinesterase ID$_{50}$ in molar concn. with acetylcholine as substrate
												Dose inj. (µg.)	Substrate			
Chemical Name	Concn. (%)	*Locusta*	*Aphis*	*Plutella*	*Sitophilus*	*Musca*	*Tyroglyphus*			ACh†	NPA‡		ACh	NPA		
Diethyl-4-chlorophenylphosphate (4-Cl)	0·3	2	3	3	0	3	3	0·54	254	2×10^{-2}	2×10^{-2}	1000	70	30	0	$7·9 \times 10^{-6}$
	0·06	—	1	0	—	2	2									
Diethyl-2-chlorophenylphosphate (2-Cl)	0·3	0	3	0	0	2	0	0·13	375	5×10^{-3}	5×10^{-3}	1000	51	31	6 in 6–9 min.	$1·3 \times 10^{-6}$
	0·06	—	3	—	—	—	—									
Diethyl-2:4-dichlorophenylphosphate (2:4-Cl)	0·3	3	3	3	2	3	3	0·078	25	$2·8 \times 10^{-4}$	$2·8 \times 10^{-4}$	100	75	89	0	$9·0 \times 10^{-11}$
	0·06	2	3	0	—	3	3									
Diethyl-2:4:5-trichlorophenylphosphate (2:4:5-Cl)	0·3	3	3	3	3	3	3	0·015	3·2	$7·5 \times 10^{-6}$	$8·7 \times 10^{-6}$	10	64	86	4 in 4–24 hr.	$5·6 \times 10^{-11}$
	0·06	3	3	2	2	3	3									
Tetraethylpyrophosphate (for comparison)	0·3	2	3	3	2	3	3	0·0031	1·5	$4·6 \times 10^{-8}$	$2·8 \times 10^{-8}$	4	86	76	MLD ~1–2 mg./kg.§	4×10^{-10}
	0·06	—	3	3	—	—	2									

* *Key:* 0 = no kill, 1 = < 50 % kill, 2 = 50–95 % kill, 3 = > 95 % kill.

† Acetylcholine chloride. ‡ *o*-Nitrophenyl-acetate. § No actual figures available. The above has been estimated as a reasonable inference from available data.

METHOD

Preparation of locust nerve cord brei

The three thoracic ganglia with their attached nerve cords were dissected out from eight to ten young adult locusts. They were carefully cleaned of all other tissue, washed in buffered saline, dried on filter-paper, and weighed. They were then homogenized with a few drops of the buffered saline and the volume of the homogenate made up with this in such a way that 1 ml. of final suspension contained 20 mg. of nerve cord. The average weight of one thoracic system was about 3 mg.

The buffered saline used was made up as follows:

	ml.
0·1 M Sorensen's phosphate mixture, pH 6·9 (made up after Clark, 1928)	2·5
20 % sodium chloride	1
1 % potassium chloride	1
3·2 % magnesium sulphate	0·25
Water to	25

All the above solutions were stored in a refrigerator.

Estimation of enzymic decomposition of o-*nitrophenylacetate* (*NPA*)

This is essentially the technique of Jansen, Nutting & Balls (1948), adapted for the present purpose.

To 0·85 ml. of buffered saline in a 7·5 × 1 cm. test-tube was added 0·05 ml. of locust nerve cord homogenate or 0·05 ml. of 1:5 dilution of normal horse serum in buffered saline. The tubes were then corked. The requisite TEPP solutions were freshly made up from 99 % pure TEPP and distilled water to 20 times the desired final molar concentration. 0·05 ml. of these were added to the test-tubes, the cork having been removed for the minimum time to prevent contamination from adjoining tubes containing other strengths of TEPP. The corked tubes were transferred to a water-bath at 37° C. for 1 hr., 0·05 ml. of 0·04 M NPA in 50 % ethanol were added and digested for the times stated in the results tables. The following control tubes were also included in each experiment: substrate and homogenate without TEPP, substrate only, homogenate only, and a tube containing 0·05 ml. of 0·04 M *o*-nitrophenol as a standard.

All tests were carried out in duplicate.

The final concentrations in the tubes were: buffer, 0·01 M; NPA, 0·002 M; TEPP as required; locust nerve cord, 1 mg./ml.; or horse serum, 1 % (0·01 ml./ml.).

When the NPA splits into *o*-nitrophenol and acetic acid, the former reacts with the sodium ions in the buffer to give the deep-yellow sodium salt. The density of the colour developed was measured in relation to the standard. For this purpose, 4·0 ml. of distilled water was added to each tube and the contents transferred to 1 cm. cuvettes and read on a 'Spekker' colorimeter with Ilford filters no. 601 or on a Unicam Diffraction Grating Spectrophotometer at 420 mμ.

To avoid as far as possible the effect of variations of activity between various enzyme preparations, one homogenisate of locust nerve cord was always divided between tests for ACh and NPA hydrolysis. Thus, Exp. A in Table 1 would be done with the same preparation as used in Exp. A in Table 2 and so on. Horse serum was always drawn sterile from the same bottle, which was stored in the refrigerator.

Estimation of the enzymic decomposition of acetylcholine (ACh)

The technique used was that described by Hestrin (1949), as modified by Diggle & Gage (1951) with some further slight modifications.

Tubes were prepared as above, except that the substrate was 0·5 ml. of a 0·04 M solution of acetylcholine chloride in 0·001 M acetate buffer, pH 4·5. Addition of this amount of acetate buffer did not affect the final pH in the tube, which remained at 6·9.

The following control tubes were included with each experiment: substrate and enzyme without inhibitor, ACh, enzyme only and buffer without any addition. All tests were made in duplicate.

After the digestion period on the water-bath, 0·2 ml. of a freshly prepared mixture of 20% hydroxylamine hydrochloride and 20% sodium hydroxide were added, the tube well shaken and allowed to stand for 1 min. Acethydroxamic acid is thereby formed from any unchanged ACh. 0·1 ml. of a mixture of equal parts of concentrated hydrochloric acid and water was then added, followed by 0·15 ml. of 10% hydrated ferric chloride in 0·1 N-HCl. A deep claret colour developed. This was compared at the 'Spekker' with an Ilford no. 603 filter or on a Unicam Diffraction Grating Spectrophotometer at 540 mμ. The density of the colour is a measure of the ACh left unchanged.

In preliminary experiments, the decomposition of ACh was measured by titrating the acetic acid produced with 0·02 N-NaOH to bromo-thymol blue in an unbuffered medium. This technique was abandoned because it was inaccurate and led to differences in the decomposition of duplicates, presumably owing to different rates of hydrolysis at slightly different pH. In addition, TEPP itself hydrolysed readily, and the acid breakdown product affected the titration. This rate of TEPP breakdown, too, seemed to vary considerably with pH.

This technique was also used to detect the enzymic hydrolysis of ethyl butyrate without inhibitor. As the development of butyric acid was too small to be measured reliably, no further work with ethyl butyrate was attempted.

General contact and stomach poison test

The following organisms were employed:

3rd- to 5th-instar hoppers of *Locusta migratoria migratoriodes* Rch. & Frm. on grass.

Aphis rumicis L. on dock leaf.

Nearly fully grown larvae of *Plutella maculipennis* Curt. on cabbage.

Adult *Sitophilus granaria* L. on wheat.

Adult *Musca domestica* L., fed on cotton-wool soaked with milk and sugar.

Tyroglyphus farinae Deg. on gelatine-glycerine jelly.

The above and their food were sprayed with a 0·3% aqueous dispersion of the chemical, containing 0·01% Agral N as wetter. Class marks are awarded for mortality as follows: 0 = kill not significantly greater than control; 1 = <50% kill; 2 = 50–95% kill; 3 = >95% kill.

Any chemical having given a class 3 response against any organism was tested again against that organism at 0·06% under otherwise identical conditions. Mortality was assessed as above.

Aphid contact toxicity

Macrosiphum pisi. (Pea aphid) adult apterous females were sprayed in the Potter Tower on filter-paper with 2 ml. of an aqueous dispersion of the test chemical at such concentrations as were found necessary to establish an LD_{50} graphically. After treatment, the insects were transferred to broad-bean leaves and mortality counts made after 24 hr. The figures in Table 5 are the means of several experiments.

Locust injection

Virgin adults of *Locusta migratoria migratoriodes* were held ventral side upwards and the size 26 needle of an 'Agla' micrometer syringe was inserted in the second abdominal tergite and pushed forward until its tip was under the mesothoracic segment. The insertion was made somewhat laterally to avoid damaging the nerve cord. All chemicals were injected in propylene glycol at a constant volume of 0·01 ml. There were usually five different concentrations after preliminary 'range-finding' experiments. Ten locusts of each sex were used for each treatment and mortality counts were made after 24 hr.

Enzyme inhibition in vivo

Twelve locusts were injected with the test chemical in propylene glycol at a constant volume of 0·01 ml. About 4 hr. afterwards when the insects showed symptoms of poisoning, the nerve cords were dissected out, washed 5 times with buffer, ground, and their activity measured in the usual way. A similar number of locusts were injected with the solvent only, and treated identically. The activity of their nerve cord brei was taken as 100% and the inhibition by the chemical calculated accordingly.

Injection of mice

Ten female mice were injected intraperitoneally with 50 mg./kg. of the test chemical in propylene glycol.

In extensive preliminary experiments suitable substrate and enzyme concentrations, duration, and pH were used and the final choice appears from the tables. In all cases, conditions were such that the dissociation of the substrate was linear over the time involved.

RESULTS

No noticeable dissociation was obtained using ethyl butyrate as a substrate and titrating with 0·02 N-NaOH to bromo-thymol blue, with locust nerve cord as the source of enzyme. It is not claimed that no splitting took place, and an electrometric method may well have shown some activity; but the hydrolysis was certainly very much smaller than with NPA or ACh, and the ethyl butyrate investigation was discontinued.

Results with TEPP as an inhibitor of the enzymic hydrolysis of NPA and ACh are given in the tables. The ID_{50}'s (i.e. the concentration of TEPP at which a 50% inhibition of enzyme activity was achieved) can be summarized as follows:

	NPA	ACh
Locust nerve	$2{\cdot}8 \times 10^{-8}$	$4{\cdot}6 \times 10^{-8}$
Horse serum	5×10^{-5}	4×10^{-10}

The figures are molar concentrations of TEPP.

Turning to the comparison of the halogenated diethylphenylphosphates, the sorting test results show the four chloro-compounds to rank in order of general toxicity, as follows: 2-Cl, <4-Cl, <2:4-Cl, <2:4:5-Cl.

This is also the order in which they rank for their effectiveness in the injection of locusts, with what would appear to be reasonable correlation. The *in vivo* inhibition of acetylesterases, which too is in that order, was based on a single dose of each chemical only, and is better disregarded. TEPP was more toxic than the chloro-compounds.

In the toxicity to aphids, however, the order of the two mono-chloro-compounds is reversed. This is in accordance with the sorting test results, where 2-Cl is shown to have a high specificity for aphids and reaches an assessment for these insects higher than any reached by 4-Cl against any organism. Compound 4-Cl derives its claim for general toxicity from its wider effectiveness. Surprisingly, however, the results for *in vitro* inhibition of locust nerve cord acetylesterase correlate with those for aphid toxicity (though on a different scale) not with those for locust injection! No outstanding differences between the activity against the two substrates appear, and from these data it would seem that the tentative suggestion that the same enzyme is responsible for the hydrolysis of both substrates has further support.

The same order of merit for the chloro-compounds appears in the inhibition of horse-serum cholinesterase with a remarkable difference: 2-Cl and 4-Cl are grouped closely together and so are 2:4-Cl and 2:4:5-Cl. A factor of about 10,000 separates the two groups! The latter two compounds are more active than TEPP, 2:4-Cl by a factor of 4 and 2:4:5-Cl by a factor of 7. All the more striking is their *in vivo* activity against mice, which shows no correlation whatsoever with the cholinesterase data: 4-Cl and 2:4-Cl are not toxic at all at this dose and 2:4:5-Cl and 2-Cl (in that order) can only be regarded as mildly toxic compared with TEPP.

DISCUSSION

All deductions drawn from these experiments must be subject to the assumption that it is sometimes justifiable to correlate *in vitro* results with *in vivo* biological activity. This assumption can however be made only in the most provisional and tentative way. A chemical may show high *in vitro* activity yet be unable to exert its influence at the site of action in the intact organism. Even though injection experiments may eliminate the problem of penetrating the complex body wall, there may still be internal barriers between the body cavity and the molecules with which the chemical reacts to give the toxic effect. Furthermore, for biological activity to be effective, it must not only be potential—the only kind shown by *in vitro* experiments—but also actually exerted at the right time and place. A chemical entering an organism may, as Diggle & Gage (1950) have shown for parathion in mammals, be changed from a non-active to an active substance before interfering with vital processes, or else be metabolized from an active to a non-active substance (detoxicated). No definite and final conclusions should therefore be drawn from the present data, though some tentative suggestions may be justified.

It is evident nevertheless from these experiments that locust nerve cord contains an enzyme capable of hydrolysing NPA, as well as one capable of hydrolysing ACh. Whether the two are, in fact, identical it is impossible to decide on present evidence, but there is certainly nothing to prove that they are not. The inhibition of both mechanisms by all the phosphoric esters tested takes place at about the same ID_{50}. A glance at the tables will show that the differences between the two are not likely to have much significance. It is also impossible to say whether the esterase (or one of them if there are two) is physiologically the same as the one detected by Lord & Potter (1951) in extracts of whole *Tenebrio* and other insects.

Be that as it may, the locust nerve cord certainly contains other enzymes susceptible to phosphoric esters than a specific acetylcholinesterase analogous to the one occurring in mammals. On the assumption that the insecticidal activity of these substances is primarily that of a nerve poison, there is no evidence to show that their action on any specific acetylcholinesterase—if such is indeed there—is their only, or even primary cause of effectiveness.

The experiments previously reported (Hopf, 1952) have thrown doubt on the proposition that acetylcholine, or for that matter, any other cholinester, plays any role at all in synaptic transmission of insects. The presence of acetylcholine in insect nerve has, to the best of the author's knowledge, never been demonstrated. It has indeed been shown (Roeder, 1948) that, following application of HETP (TEPP) or eserine, *Periplaneta* nerve cord contains a substance having an effect similar to that of ACh on frog nerves. It does not follow from this that ACh is the synaptic mediator, and Roeder stresses this point.

We are left with the position that, although insect nerve cord does indeed hydrolyse ACh, the enzymic activity is by no means restricted to this, nor is the activity of phosphoric insecticides. At the present state of our knowledge of these enzymes or enzyme—and excluding the argument by analogy from mammals to insects—the fact that insect nerve cords hydrolyse ACh seems as irrelevant to the physiological function of the enzyme or the inhibition involved as the fact that they hydrolyse *o*-nitrophenylacetate, for which nobody has claimed a normal physiological function. In these circumstances the terms cholinesterases or even acetylcholinesterases applied to insects may be misleading. Deacetylation is one of the most common physiological processes and the fact that two acetylesters were split, whereas no success was obtained with ethyl-butyrate seems to indicate that some such process is involved. Consequently, the enzyme or enzymes in question might provisionally be termed simply acetylesterase(s) leaving open the question whether one or more are involved.

As for horse serum, there seem to be two distinct enzymes as shown by the different level of TEPP inhibition. These enzymes are clearly of a type unrelated to those of locust nerve. The high susceptibility of the cholinesterase underlines the grave risks involved in handling TEPP. Horse serum is about 100 times as sensitive to this substance as locust nerve. Grob (1950) reports an ID_{50} for TEPP of 5×10^{-9} M for the cholinesterase of human plasma, Dubois, Doull, Salerno & Coon (1949) $8{\cdot}6 \times 10^{-10}$ M for the same. The findings therefore appear to be reasonable.

While no inference from the behaviour of serum to that of mammalian nervous tissue should be drawn, this technique may well prove very useful as a tool in the search for new insecticides, particularly organic phosphorus compounds, of low mammalian toxicity. The evidence of the present work clearly points to the *possibility* of the existence of such compounds, as the physiological differences between the insect and mammalian nervous system become clearer.

It is, of course, dangerous to draw too many conclusions from the non-specific cholinesterase of horse serum to the behaviour of the acetylcholinesterase, the inhibition of which is assumed to be the cause of toxicity of TEPP, E 600, and other substances in mammals. But, on the assumption that such conclusions can be drawn, one possible explanation of the lack of correlation between the two series of mammalian results suggests itself. The mammal has a detoxication mechanism that can deal effectively *in vivo* with substances like 2:4-Cl and 2:4:5-Cl. What properties in the inhibitor molecule lend themselves to action by this mechanism, cannot be surmised on present data. This detoxication mechanism cannot affect TEPP or E 600, both of which are powerful *in vivo* inhibitors and also highly toxic by injection, nor, presumably the metabolite of parathion which is responsible for the activity of that chemical.

Some additional support for the presence of the detoxication mechanism is provided by the fact that all survivors in these experiments recovered quickly from the symptoms (lethargy) suffered after treatment. There is no suggestion of such a detoxication mechanism in the insect (on the basis of the present data).

There are very strong indications that the inhibition of the acetylesterases of the nerve cord is not the only cause of toxic action by these substances in insects. 2-Cl inhibits the enzyme (or enzymes) more effectively in locusts nerve cord *in vitro* than 4-Cl, but the latter is more toxic by injection. Furthermore, there are the experiments on *in vivo* inhibition of the acetylesterases by injection of the test-compounds. In mammals it is generally known that a 90% inhibition of acetylcholinesterase is necessary before toxicity is effective. The present substances were injected at 3–4 times the LD_{50} yet 90% was not obtained even by TEPP. The percentage inhibition is not likely to be more than the percentage killed; it may be lower. However, the insects were not dead at the time of dissection. In order to avoid post-mortem changes as a source of error, the time chosen was that when symptoms appeared well established, but before death. It is possible that at a later time inhibition would have been more pronounced.

An interesting clue on selective toxicity to various types of insects is provided by these data. The order of toxicity of 2-Cl and 4-Cl to *Macrosiphum pisi* is the same as that of inhibition of locust nerve cord acetylesterases. One is led to the deduction that action on mechanisms other than the inhibition of these enzymes is the primary cause of death in *Locusta* and the other insects against which 4-Cl showed higher toxicity, whereas in aphids acetylesterase-inhibition is probably the main factor of toxicity. Could it not be that non-selective phosphoric insecticides, like three of the present chlorophenyl phosphates, have two entirely distinct effects on the physiology of the insects: an inhibition of the acetylesterase of the nerve cord (and possibly similar enzymes in other parts of the body) and another one, completely unknown at present, but more important against most insects, while compounds of the type of 2-Cl possess only the former property?

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THE SYSTEMIC INSECTICIDAL ACTION OF CERTAIN COMPOUNDS OF FLUORINE AND OF PHOSPHORUS ON *PHAEDON COCHLEARIAE* FAB.

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Four compounds—*bis*dimethylaminophosphonous anhydride (*schradan*), *bis*(dimethylamino)fluorophosphine oxide (*dimefox*), diethyl paranitrophenyl phosphate (*paraoxon*) and sodium fluoroacetate (*acetate*)—have been tested against the mustard beetle, *Phaedon cochleariae* Fab. When the four compounds are compared by a direct contact technique in which insects can also imbibe some of the test solutions, or the insects are caged with foliage which has been dipped and then dried, the order of toxicity to adults and larvae was found to be

Paraoxon > acetate ≏ dimefox > schradan.

The paraoxon is very much more toxic than the other compounds. In all cases the adults are more resistant than the larvae.

When the insecticides are applied systemically the order of toxicity changes to

Paraoxon > dimefox > acetate ≏ schradan

because the acetate causes the plants to wilt and less of it is absorbed. Both paraoxon and dimefox give complete kills of adult *Phaedon* which is the most resistant stage, at practical concentrations.

When the insecticides were applied to the outer leaves and translocated to the inner untreated leaves an effect was observed with the dimefox and paraoxon after numerous application, but with a single application very inconsistent results were obtained.

It is concluded that schradan and the acetate are ineffective systemically against *Phaedon*. The dimefox and paraoxon are very effective, but because of their high mammalian toxicity they would require very careful handling in practice.

When it became known that certain systemic insecticides controlled aphids effectively, it seemed desirable to investigate their action on other insects feeding on plants. An earlier paper has described the action of four compounds on the eggs and larvae of *Pieris brassicae* L. (David & Gardiner, 1953), and the present investigation concerns the effect of these compounds on the eggs, larvae and adults of the mustard beetle, *Phaedon cochleariae* Fab.

Materials tested

The materials used in this investigation were (i) *bis*dimethylaminophosphonous anhydride here called *schradan*, but previously called *anhydride*; (ii) *bis*(dimethylamino)fluorophosphine oxide, here called *dimefox* and previously *oxide*;

(iii) diethyl *p*-nitrophenyl phosphate, here called *paraoxon* and previously E 600; and (iv) sodium fluoroacetate, called the *acetate*. The properties and origins of these compounds have already been described (David & Gardiner, 1951).

Methods and equipment

(1) *The greenhouse*. The insect culture was kept, and the experiments were conducted, in the greenhouse previously described (David & Gardiner, 1951).

(2) *The test insects and plants*. The mustard beetle was chosen to represent the coleopterous plant-feeding insects, because it is convenient to handle and can be bred in the laboratory all the year round (Way, Smith & Hopkins, 1951). The beetles were reared on turnip plants in 3½ in. 'Whalehide' pots containing John Innes pasteurized potting compost. Young plants were used in which the stem had not started to swell. To obtain eggs adult insects were confined on the plants in hurricane lamp glasses covered at the top with muslin. Since the eggs hatch only on living leaves it is essential to change the plants on which the adults are laying before the leaves are too much eaten. The adults were transferred conveniently and rapidly but turning the pot and lamp glass together on to their sides and then jarring the pot against the edge of the bench. The adults dropped off into the lamp glass and the first plant was then withdrawn and replaced by a fresh one. A similar procedure was used with the larvae, except that they were not shaken off the old plant but this was uprooted instead and transferred within the lamp glass to a fresh potted turnip. This process was repeated several times, and when the larvae were almost fully fed they were placed with cut turnip leaves on a layer of moist sandy compost in a 4–6 in. crystallizing dish covered with muslin. Fresh leaves were added daily until all the larvae had disappeared into the soil to pupate. When kept at an average temperature of 20° C. the adults reached their maximum rate of egg production when about 3 weeks old and the eggs then hatched in about 6 days.

In winter the turnip plants were kept in a cool greenhouse and given extra light from a fairly powerful source such as a sodium vapour lamp.

(3) *Cages*. During the tests it was necessary to confine the insects, and as they tended to wander away from the plants it was found advisable to keep the cages as small and simple as possible. In the type eventually adopted the petiole of a single leaf was arranged to pass through a narrow slot 1 in. deep in a thin strip of hardboard. Several of these slots were cut at 5 in. intervals along the length of the strip. After arranging the plants in position the remainder of each slot was blocked with cotton-wool or sponge rubber. Glass rings 3¼ in. in diameter and 1½ in. high (cut from 2 lb. jam jars) were then placed round the leaves on filter-papers slit to take the petioles. To complete the cages the tops were closed either with Perspex plates perforated with several holes covered with bolting silk or with pieces of muslin held on by rubber bands.

Assessment of the effect of the materials on the insects and plants

Several attempts were made to determine the ovicidal properties of the compounds, but this proved to be impracticable for reasons which will be considered later. In these tests, however, a sufficient number of eggs hatched for observations to be made on the dosage of insecticide likely to be toxic to the newly emerged larvae (Table 2).

In the tests with 2nd-stage larvae and adults a known number of insects, usually ten, was placed on each of the test plants, which had been dipped or treated systemically, and the percentage killed was determined daily up to 5 days and sometimes longer. The final results are based on a total of about forty to eighty insects in each case. Deaths among the controls were negligible. Any insect capable of the slightest movement was counted as alive.

In certain cases the phytotoxicity of the insecticide solutions was assessed and the results are expressed in the tables as follows:

Normal	N	Not differing from controls.
Trace	T	Slight wilting or marginal leaf scorch or scattered spotting.
Moderate	M	Extensive wilting and/or large areas of the leaves scorched and some entirely destroyed.
High	H	Plant almost entirely withered though the youngest undeveloped leaves may be green.
Dead	D	Plant dead.

Results

(1) *Direct contact and stomach poison action*

The first series were dipping tests, in which there was a direct contact with the insecticide and the possibility that the insect would swallow some solution at the same time. For these tests the 2nd-stage larvae or adults, according to the experiment, were counted in batches of ten and held in 3 × 1 in. tubes. Solutions of the insecticides at 20° C. containing 0·1 % (v/v) Teepol as a wetting agent, were poured on to them, and after 5 sec. the insects were decanted on to a strainer and transferred to a filter-paper and from here to the glass ring cages already described. The larvae, but not the adults, were carefully placed on the turnip leaf within the cage. The number of dead was counted daily for 5 days or longer, and the surviving insects were given a fresh leaf to feed on when necessary.

The results obtained in these experiments are shown in Table 1, from which it can be seen that paraoxon is outstandingly more toxic than the other three compounds. It will be noted that the contact toxicity of schradan is negligible at 1 %.

In the second series of tests, besides the direct contact action on the egg, the larvae feed on insecticide-dipped foliage and spend most of the time in contact with it. For these tests adults at the peak of their laying period were enclosed with well-developed turnip plants for 2 days. Even when the number of insects and the number and sizes of the leaves on which they were to lay were controlled it was not possible to collect the necessary number of eggs before the leaves had been appreciably eaten by the adults. It must be assumed that the damaged state of the leaves will affect both accumulation of insecticide during dipping and subsequent translocation.

Table 1. *Mortality of larvae or adults immersed in solutions of the insecticide and fed on untreated foliage*

		Percentage kill			
		2nd-stage larvae		Adults	
Material	Concentration (%, v/v)	2nd day	5th day	2nd day	5th day
Schradan	1·0	0	10	0	0
	0·2	0	0	—	—
Dimefox	0·5	0	0	35	55
	0·2	0	0	—	—
Acetate	0·1 (w/v)	0	0	0	5
Paraoxon	0·05	100	—	95	100
	0·01	90	95	70	100
	0·001	5	5	0	0
Control	—	0	0	0	0

When the eggs were between 2 and 3 days old the leaves bearing them were dipped individually in a shallow dish of the insecticide at 20° C. to which 0·1% 'Teepol' had been added as a wetting agent. The plants were then allowed to dry on their sides and kept in the greenhouse at about 17–23° C. until 5 days after the larvae had hatched. At this time the numbers of larvae and hatched eggs were counted. During these 5 days some normal larvae may have dropped off the plant and some of the egg-shells may have been eaten by the larvae, though the counts on the control plants did not indicate that these were important sources of error.

In the third series of tests the leaves of the test plants were dipped in the insecticides as described for the egg-bearing plants in the last section, and the larvae or adults were then caged in the glass ring cages with the treated leaves so that they could accumulate insecticides by contact and as a stomach poison.

The results of these experiments are shown in Table 2. Paraoxon was very much more toxic than the other compounds, the order of toxicity being

Paraoxon > acetate ≃ dimefox > schradan.

The schradan showed very low toxicity to the 2nd-stage larvae and adults, but at 1% it killed newly emerged larvae. The adults were slightly more resistant than the larvae. When the kill of larvae or adults reached 50% or more by the 5th day it was usually found that less than 25% of the leaf was eaten. In the case of treatment resulting in a low kill more than 75% of the leaf was consumed in this time. Only paraoxon at 0·05% caused some leaf scorch.

Table 2. *Percentage kills of larvae emerging from eggs dipped on turnip leaves and of larvae and adults fed on dipped foliage*

		Percentage kill				
			2nd-stage larvae		Adults	
Material	Concentration (%, v/v)	Young larvae, 5th day	2nd day	5th day	2nd day	5th day
Schradan	1·0	100	—	—	0	10
	0·5	—	30	85	—	—
	0·2	—	5	70	0	0
	0·1	0	0	0	—	—
Dimefox	0·5	0	—	—	0	90
	0·2	0	90	100	5	50
	0·1	—	—	—	0	25
	0·05	—	0	38	0	10
Acetate	0·1 (w/v)	50	40	100	0	15
	0·01	0	5	25	0	0
Paraoxon	0·05	100	—	—	—	—
	0·01	0	82	100	70	100
	0·005	—	68	100	—	65
	0·001	—	—	—	10	0
Control	—	—	0	0	0	0

(2) *Systemic action following absorption by the roots*

(*a*) *Plants with roots in solutions.* The young turnip plants were unpotted and their roots were washed carefully before transferring to the insecticide solutions (David & Gardiner, 1951). When the plants bore eggs the roots were placed in the solutions 3 days before the eggs were due to hatch. When larvae or adults were to be fed on them the treatment began 2 days beforehand. The results are given in Table 3.

(*b*) *Plants in soil.* In all tests 20 ml. of the insecticide solutions containing the required dose of insecticide were watered on to the 400 g. of potting compost in which each plant was growing. The pots stood in Petri dishes and were subsequently watered in such a way that there was no drainage from the bottom of the pots (David & Gardiner, 1951). The results are given in Table 4.

As the results of the experiments with the plants in solution and in potting compost are similar they may be considered together. Wherever the data are sufficiently complete to allow comparisons to be made it is found that the order of toxicity of the four compounds is:

Paraoxon > dimefox > acetate ≃ schradan.

There is only one exception to this, namely, in the case of plants in solutions of the schradan and acetate on which 2nd-stage larvae were feeding. This is in fact only an apparent change in the order of effectiveness. It can be explained by the observation that the acetate often caused turnip plants to wilt, and this was shown in the

TABLE 3. *Toxicity records on turnip plants with roots in insecticide solutions: (a) egg-carrying plants with roots placed in solutions 3 days before egg hatch; (b) plants with roots placed in solutions 2 days before the feeding of larvae or adults on the foliage*

			2nd-stage larvae			Adults			
		Young larvae, percentage kill on day	Percentage kill on day		Percentage leaf consumed by day 5	Percentage kill on day		Percentage leaf consumed by day 5	Leaf damage, 5th day
Material	Concentration (%, v/v)	5	2	5		2	5		
Schradan	0·5	—	10	100	<25	0	100	>75	T
	0·1	—	10	100	—	0	0	>75	N
	0·05	100	5	25	—	—	—	—	N
	0·01	0	0	0	>75	—	—	100	N
Dimefox	0·05	—	100	—	<25	95	100	<25	M
	0·01	—	75	100	<25	50	75	<25	T
	0·005	100	—	—	—	—	—	—	—
	0·001	—	60	95	<25	10	55	<25	T
	0·0005	75	—	—	—	—	—	—	—
	0·0001	—	5	25	>75	0	0	>75	N
	0·00005	0	—	—	—	—	—	—	—
Acetate	0·1 (w/v)	—	0	20*	<25	20	100	<25	M
	0·05	100	0	0	<25	0	35	>75	M
	0·01	—	—	—	—	0	0	100	T
	0·005	0	—	—	—	—	—	—	—
Paraoxon	0·01	—	100	—	<25	100	—	<25	M
	0·001	—	100	—	—	80	100	<25	T
	0·0005	100	—	—	—	—	—	—	—
	0·0001	—	0	40	>75	0	50	<25	N
	0·00005	0	—	—	—	—	—	—	—
Control	—	0	0	0	100	—	—	100	N

* These plants had wilted, see text.

TABLE 4. *Effect of watering the insecticide solutions (20 ml.) on to the soil in the pots in which turnip plants were growing. In tests against young larvae the solutions were applied 6–8 days before the eggs hatched; in tests against 2nd-stage larvae and adults, 2 days before the insects were put on the plants*

			2nd-stage larvae			Adults		
		Young larvae, percentage kill on day 5	Percentage kill on day		Percentage leaf consumed by day 5	Percentage kill on day		Percentage leaf consumed by day 5
Material	Concentration (%, v/v)		2	5		2	5	
Schradan	1·0	100	—	—	—	—	—	—
	0·5	0	0	15	100	0	0	100
	0·2	—	0	0	100	—	—	—
Dimefox	0·1	100	—	—	—	100	100	<25
	0·05	—	100	—	<25	80	100	<25
	0·01	—	90	100	<25	65	95	<25
	0·005	—	25	100	<25	—	—	—
	0·001	80	5	75	<25	0	10	33
Acetate	0·5 (w/v)	100	0	0	100	0	0	100
	0·1	80	—	—	—	—	—	—
	0·01	0	—	—	—	—	—	—
Paraoxon	0·05	100	100	—	<25	100	—	—
	0·01	0	100	100	<25	95	100	<25
	0·005	—	30	100	—	0	60	<25
	0·001	—	0	5	100	0	5	100
Control	—	0	0	0	100	0	0	100

case of plants in solution to be associated with a reduction in the volume of acetate solution taken up by the plant (Table 5).

It will be noted that when applied systemically dimefox is much more effective than the acetate, whereas in the dipping tests they were about equal. Schradan remains the least toxic of the four compounds.

When the 2nd-stage larvae are compared with the adults the latter are found to be rather more resistant. The newly emerged larvae are, with one exception (in a test using paraoxon), more susceptible than 2nd-stage larvae to the insecticides applied systemically.

TABLE 5. *Uptake of solution by turnip plants in solutions of the schradan and acetate, and in water. Each value is the average of four experiments*

Material	Concentration (%)	Percentage volume of solution absorbed on day 2	4
Schradan	0·1	44	77
Acetate	0·1	36	44
Water	—	44	76

During the experiments observations were made on the quantity of leaf consumed. It was found that if the insecticides gave a high kill on the 5th day they did so when less than 25% of the leaf area had been consumed, i.e. about 1–1·5 sq.in. by ten insects. Only when the acetate was tested on larvae was there any evidence that insects were deterred from eating without being killed.

No phytotoxic effects were observed on the plants treated in soil, except with the acetate at 0·5%, but plants treated in solution showed scorching and wilting at the higher concentrations as shown in Table 3.

Besides the foregoing experiments, which were terminated after 5 days, others were carried out which were continued either for 15 days, starting with late 1st-stage or early 2nd-stage larvae, or for 20 days, using adults. The experiments with larvae often came to an end between the 10th and 15th day because the insects pupated. The procedure was in all other respects the same as that used in the foregoing experiments.

The object of these tests was to discover whether toxic effects that had been missed in the short-term experiments became apparent after prolonged feeding on treated plants. This information was particularly sought in the case of schradan but there was no evidence of a delayed effect (Table 6).

Very little of the leaves of the plants treated with the acetate were eaten, but the ten insects in each cage consumed several leaves of the plants treated with the other insecticides.

(3) *Systemic action following absorption by the leaves*

In this experiment three or four well-developed outer leaves of each young potted turnip plant were brushed with solutions of the insecticides containing

0·1 % Teepol, leaving two small inner leaves and the unexpanded leaves of the growing point untreated. These inner leaves on which the larvae were fed were surrounded by a shallow cone of thin cardboard, hollow side uppermost, on which a lamp glass rested. Together they screened the untreated leaves from treated parts of the plant. A slow stream of compressed air was blown into the lamp glass. Thus it was possible to prevent the insecticide applied to the outer leaves reaching the larvae except by translocation through the plants.

Table 6. *Long-term experiment. Effect of the insecticide solutions (20 ml.) watered on to the soil in which the turnip plants were growing. The plants were treated 2 days before the insects were placed on them*

		Larvae, percentage kill on day			Adults, percentage kill on day			
Material	Concentration (%, v/v)	5	10	15	5	10	15	20
Schradan	0·5	40	65	P	0	0	0	0
	0·2	5	10	P	0	0	0	0
Dimefox	0·001	100	—	—	0	5	5	15
	0·0001	35	75	100	0	0	0	0
Acetate	0·5 (w/v)	—	—	—	0	0	+	+
Paraoxon	0·001	0	0	P	0	0	5	5
Control	—	0	5	5	0	0	0	0

P = remaining insects pupated; + = plants too much wilted to continue the test.

Table 7. *Records of systemic action following applications of solutions of the insecticides to the outer leaves of turnips on larvae of the mustard beetle feeding on the inner leaves which were screened in a ventilated lamp glass*

			Treatments		Leaves		Percentage kill on day		Damage to treated leaves
Material	Concentration (%, v/v)	Date	No. given	Days*	No. treated	No. untreated	2	5	
Schradan	0·2	20. ii. 52	9	2½	3	2	0	0	N
Dimefox	0·2	4. x. 52	16	4	4	2	60	100	N
		11. ii. 53	15	4	4	2	100	—	N
	0·1	20. ii. 52	9	2½	3	2	35	100	N
		11. ii. 53	15	4	4	2	100	—	N
Acetate	0·2 (w/v)	20. ii. 52	9	2½	3	2	0	0	T
Paraoxon	0·1	11. ii. 53	15	4	4	2	80	100	M
Control	—	20. ii. 52	9	4	4	2	0	0	N
		11. ii. 53	15	4	4	2	0	0	N

* Number of days on which the treated leaves were brushed.

Following the procedure described it was found that after repeated applications sufficient quantities of dimefox and paraoxon were translocated through the plant to kill the larvae on untreated leaves. The acetate and anhydride were ineffective (Table 7).

When the above experiment was repeated using a single application of the dimefox or paraoxon by brushing, extremely inconsistent results were obtained both between replicates within an experiment and between different experiments. This occurred with carefully selected plants in which the areas of the treated leaves and of untreated leaves were kept as uniform as possible. Thus in some experiments a 0·2% solution of the dimefox applied to the outer leaves killed 100% of the larvae feeding on the untreated inner leaves, while in others a 0·5% solution used in the same way killed under 20% of the larvae.

DISCUSSION

In the earlier part of the paper it has been explained that it was not possible to determine whether any of the compounds prevented the emergence of larvae from the eggs, and this problem may now be considered further. Unlike *Pieris* eggs, those of *Phaedon* will not normally hatch if the leaf on which they are laid has dried up, though on close examination it is found that the cells in the layer immediately adjacent to the egg are always dead—no doubt having been damaged when the female cut the cavity for the egg. It has been shown by Way *et al.* (1951), however, that if the relative humidity is above about 86% *Phaedon* eggs will hatch on dried leaf tissue. These two observations together suggest that the *Phaedon* egg depends on the living leaf tissue to maintain a high relative humidity around it but that neither water nor toxic material would reach the egg across the layer of dead plant cells.

TABLE 8. *The approximate amounts of the four compounds required to give complete kills of* Aphis, *or* Pieris *third instar larvae and* Phaedon *adults*

	Methods of dosing					
	By dipping, percentage solution			Systemically from soil, c.c. per pot*		
Material	*Aphis*	*Pieris*	*Phaedon*	*Aphis*	*Pieris*	*Phaedon*
Schradan	0·05	>0·2	>1·0	0·02	>0·04	>0·10
Dimefox	0·05	>0·1	>0·5	0·002	>0·02	0·01
Acetate	0·001	>0·1	>0·1	0·001 (g.)	0·02 (g.)	>0·1 (g.)
Paraoxon	0·0005	0·01	0·01	>0·04	0·002	0·002

* $3\frac{1}{2}$ in. diameter pots containing about 400 g. compost.

In the experiments, adults in full egg production were placed on the turnip plants. It was found that they had to be left on the plant 24 hr. to lay a sufficient number of eggs to give a reliable test. In this time, however, the insects ate an appreciable area of the leaves and as a result further areas withered. Thus at the time of hatching, too few eggs were left embedded in living tissue to make a count worth while. This same factor interfered with the tests with newly hatched larvae, and the differing extent and localization of the damage was doubtless responsible for the variability in the results of these tests.

In order that the relative effectiveness of the four compounds against *Aphis fabae*, *Pieris brassicae* and *Phaedon cochleariae* may be easily appreciated, the results obtained in this and earlier papers have been summarized in Table 8.

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STUDIES OF THE TOXICITY TO WORKER HONEY-BEES (*APIS MELLIFERA* L.) OF CERTAIN CHEMICALS USED IN PLANT PROTECTION

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(With 2 Text-figures)

Laboratory techniques are described for the estimation of the stomach poison, direct and residual film contact poison and fumigant poison effects of chemicals to adult worker honey-bees.

The toxicity of eleven chemicals used in plant protection has been investigated by these methods. The order of effectiveness as stomach and contact poisons was: parathion, TEPP, γ-BHC, dieldrin, aldrin, chlordane, *o*, *o*-diethyl-*o*-ethylmercaptoethyl thiophosphate (constituent of Systox), bisdimethylamino fluorophosphine oxide, toxaphene and the sodium salts of 2:4-D and MCPA: as residual films, dieldrin, aldrin, γ-BHC, parathion, chlordane, and *o*, *o*-diethyl-*o*-ethylmercaptoethyl thiophosphate (constituent of Systox); toxaphene, TEPP and bisdimethylamino fluorophosphine oxide had no measurable effect; as fumigants, dieldrin, γ-BHC, aldrin, parathion, and chlordane; the remainder had no measurable effect.

This investigation was undertaken to ascertain whether field applications of a particular chemical as an insecticide, fungicide or herbicide would prove harmful to foraging honey-bees. Work of this nature has been done in various countries, but, often for reasons indicated by Eckert (1949) and Hafliger (1949), the results obtained by the various research workers are not comparable.

In this paper a description of techniques used in evaluating the toxicity of a compound is given, and the results of applying these techniques to eleven chemicals are discussed in relation to other published information.

Selection of test insects

Six colonies of Italian hybrids were used throughout the tests. The bees were obtained by placing a large glass jar over the feed hole and tapping the hive. A stream of bees would pass into the jar, and these were removed by sliding a piece of perforated zinc between the jar and crownboard. All bees were capable of strong flight and most were over 13 days old. The method of collection did not necessitate any handling of the bees and prevented any mortality from physical injury. No evidence has appeared in the literature to suggest that different strains of *Apis mellifera* vary in their resistance to poisonous chemicals, and it was assumed that each sample of bees was fairly uniform. No evidence to the contrary was collected during the tests.

POST-TREATMENT PROCEDURE

After treatment, the bees were placed in cages made of perforated zinc (Glynne Jones & Edwards, 1952), five bees in each cage. These were kept in a constant temperature/humidity cabinet at 30° C. and a relative humidity of 55–60%. The bees were given unrestricted access to a 50% sucrose solution. The mortality was recorded at intervals for periods up to 72 hr., but as most of the deaths occurred within 24 hr., it was decided to make comparisons at this time. This may be justified further on the grounds that the environment in the cage is abnormal and defaecation is inhibited. Moribund bees not actually dead were included in the total kill recorded.

TECHNIQUES USED IN THE BIO-ASSAY

Stomach poison tests. The following technique was used to feed individual doses to bees. A large sample of workers (at least fifty) was collected and kept in an active state at 30° C. for at least 1 hr. to induce 'hunger', then anaesthetized with carbon dioxide. Individual bees were placed in small glass tubes, abdomen first (Fig. 1), and a measured dose (generally 0·02 ml.) of the test chemical made up in a 50% sucrose solution was introduced into the small-bore glass tube in the cork with an Agla micrometer syringe; weighing tests showed that with a volume of 0·02 ml. the variation in the amount introduced was less than 2%. After 30 min. any bees which had regurgitated the liquid or had not recovered properly from anaesthesia were discarded, and the rest, normally two-thirds of the bees placed in the tubes, were subjected to the post-treatment procedure outlined above. Control bees were treated

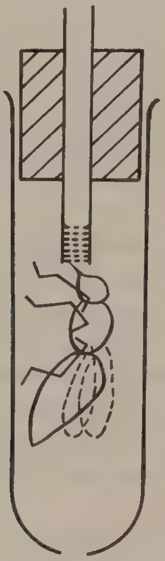

Fig. 1. Diagram of apparatus used to feed individual doses to bees.

in a similar manner. In previous work (Glynne Jones & Edwards, 1952) it was considered necessary to cage treated bees individually to prevent them feeding each other and hence altering the dose per bee. However, it was found that, provided the bees were starved prior to treatment, the volume of the honey stomach contents was fairly uniform and individual caging was not considered essential.

Contact poison tests (*aqueous solution*). The apparatus used was a replica of the improved laboratory spraying apparatus incorporating the final design of atomizing nozzle as described by Potter (1952).

The data on the weights of a series of replicate deposits obtained by spraying 5 ml. of distilled water on a 9 cm. Petri dish are given in Table 1. The variation in the weight of the deposit was small and the results compare favourably with those obtained by Potter with the original apparatus.

TABLE 1. *Replication of spray deposit of distilled water on a 9 cm. Petri dish*

(Tower temperature, 10° C.; R.H., 70%; air pressure of atomization, 24–25 cm. Hg; volume of fluid in reservoir, 5 ml.; time taken to spray, 13 sec.)

Test no....	1	2	3	4	5	6	7
mg./dish	442·2	484·0	470·0	480·0	478·5	486·0	463·0
Test no....	8	9	10	11	12	13	
mg./dish	448·0	472·5	464·0	488·0	467·5	498·0	

Mean total deposit = 472 ± 4·4 mg. S.D. = ± 15·9 mg.

The procedure in a test was as follows: thirty bees were anaesthetized with carbon dioxide and evenly distributed on a 9 cm. Petri dish lined with filter-paper. Immediately after spraying, the bees were transferred to cages. Control bees were sprayed with distilled water containing the same wetting agent as in the test spray. The weight of the deposit per sq.cm. was obtained by averaging the weight of deposit obtained on a glass plate before and after each series of sprayings. Further tests showed that on average a deposit of 7·6 mg./sq.cm. of aqueous spray corresponded to 5·3 mg. per bee. Small differences would occur when different wetting or emulsifying agents were used. The caged bees were subjected to the post-treatment procedure described previously.

Dry-film test. This test measured the effect of maintaining bees in contact with a dry film of the test chemical for 1 hr. This time was selected as it approximates to the maximum time that a foraging bee is likely to be in actual contact with treated foliage in any one day.

An air-brush (Aerograph model N.S.A.) was used to spray a measured amount of the test chemical in an aqueous medium evenly on a known area of a non-adsorbent paper (household grease-proof paper). This was used to line all the inside surface of a circular cage 12 cm. high and 10 cm. in diameter, made of perforated zinc with holes pierced in the paper lining to effect ventilation. A fuchsin dye was incorporated with the chemical and this permitted of a visual check of the evenness of the deposit. A gentle stream of air was blown into the cage so as to prevent any build-up of the chemical in the vapour phase, and it had the additional effect of stimulating the bees to run actively over the treated surface.

The cage was kept in the constant temperature cabinet at 30° C., and up to fifty bees were introduced at each treatment. After 1 hr. the cage was placed in an atmosphere of carbon dioxide to anaesthetize the bees. This facilitated transference to the post-treatment cages, which were maintained under the same conditions as in the previous tests. No food was supplied during exposure but the bees were fed before treatment. Originally, attempts were made using plant leaves to line the cage thus simulating the residual films encountered under field conditions. Unfortunately this was found to be impracticable owing to the difficulty of lining the cage with leaves.

TABLE 2. *Active materials and formulations*

Material	Method of preparation in aqueous medium
Parathion: redistilled sample of *o*, *o*-diethyl-*o*-*p*-nitrophenyl thiophosphate	Emulsion prepared by shaking with water containing 0·03 % (w/v) sulphonated lorol
Dieldrin: pure sample, m.p. 173° C.	Dissolved in minimum of benzene and an aqueous emulsion prepared using 0·05 % (w/v) cyclohexylamine dodecyl sulphate. Less than 0·2 % (v/v) benzene in final dilution.
Chlordane: technical grade	
Aldrin: pure sample, m.p. 102° C.	
Benzene hexachloride: technical sample of γ-isomer	
Toxaphene: commercial 50 % dispersion	Dispersion shaken with water and 0·03 (w/v) sulphonated lorol
Tetraethyl pyrophosphate: commercial sample containing 40 % (w/w) active ingredient	Aqueous solution; 0·03 % (w/v) sulphonated lorol added
Bisdimethylamino fluorophosphine oxide (dimefox): pure compound	
Sodium salt of 2-methyl-4-chlorophenoxyacetic acid: pure compound	
Sodium salt of 2:4-dichlorophenoxyacetic acid: pure compound	
o, *o*-Diethyl-*o*-ethylmercaptoethyl thiophosphate (constituent of Systox) with 50 % (w/v) emulsifying agent of polyoxyethylene alkyl aryl phenol type	Aqueous emulsion

Tests for fumigant action. To obtain information as to the relative effectiveness of the test chemicals as fumigants, modifications were made in the apparatus used for the dry-film tests. The treated paper was not perforated before it was used to line the cage and the fifty bees were placed in an inner cage of perforated zinc which was separated from the paper by 0·5 cm. in all directions. No air was blown into the cages. In this way, without having direct contact with the treated surface, the bees were maintained in an atmosphere which permitted the build-up of the chemical in the vapour phase.

The bees were treated for 1 hr., and after the introduction of a glass U-tube to permit feeding with sucrose solution, the whole cage was transferred to the post-treatment cabinet.

Field tests. No satisfactory technique has yet been found for field tests. Some experiments were made using a portable bee-proof cage measuring 12 × 12 × 6 ft. This, together with a small nucleus hive, was placed on plots of mustard as they came into flower. The plants were treated when the flowers became attractive to bees. It was found impossible to obtain significant results using such a small area, and it was considered that a cage at least 6 times larger was the minimum required. Weaver (1950) obtained useful results using a cage covering 1296 sq. ft.

RESULTS

The relationships between dose and toxicity established by the feeding and spraying tests are shown graphically in Fig. 2. The percentage kill in probits was plotted against log dose and the regression line fitted by eye (Finney, 1947). The lines were used to estimate the expected doses required to produce kills of 20, 50 and 90% (Tables 3 and 4). The results of the dry film (Table 5) and fumigation tests (Table 6),

TABLE 3. *Probit analysis of data from stomach poison tests with the calculated doses required to give* 20, 50 *and* 90% *kill in* 24 *hr.*

Material	Regression equation	Lethal dose (mg. × 10^{-5})		
		20%	50%	90%
Parathion	$Y=3{\cdot}54+2{\cdot}36x$	1·8	4·0	14·4
TEPP	$Y=8{\cdot}16x-1{\cdot}69$	5·2	6·5	9·3
*γ-BHC	$Y=2{\cdot}00x+3{\cdot}20$	2·6	7·9	34·6
Dieldrin	$Y=10{\cdot}60x-10{\cdot}16$	22·3	26·9	35·4
Aldrin	$Y=7{\cdot}00x-4{\cdot}66$	18·1	23·9	36·5
Chlordane	$Y=6{\cdot}80x-8{\cdot}94$	83·1	112·2	173·0
Systox	$Y=12{\cdot}14x-21{\cdot}34$	125·6	147·8	188·4
Dimefox	$Y=4{\cdot}61x-5{\cdot}51$	125·0	190·5	350·6
Toxaphene	$Y=4{\cdot}21x-10{\cdot}16$	2,512·0	3,981·0	8,017·0
2:4-D salt	$Y=5{\cdot}09x-15{\cdot}46$	7,145·0	10,450·0	18,660·0
MCPA salt	$Y=8{\cdot}54x-28{\cdot}97$	8,318·0	10,470·0	14,830·0

* These figures were calculated from the data given by Way & Singh (1948).

TABLE 4. *Probit analysis of data from contact poison tests (aqueous spray) with the calculated doses required to give* 20, 50 *and* 90% *kill in* 24 *hr.*

Material	Regression equation	Lethal dose (mg./sq.cm. × 10^{-5})		
		20%	50%	90%
Parathion	$Y=6{\cdot}08x-4{\cdot}42$	25·7	35·4	57·4
TEPP	$Y=8{\cdot}88x-9{\cdot}65$	35·8	44·5	62·1
Dieldrin	$Y=4{\cdot}88x-3{\cdot}59$	38·6	57·5	105·2
Aldrin	$Y=3{\cdot}61x-1{\cdot}32$	32·7	56·2	127·4
γ-BHC	$Y=20{\cdot}00x-33{\cdot}60$	77·2	85·1	98·6
Chlordane	$Y=7{\cdot}09x-14{\cdot}14$	380·2	500·0	758·0
Systox	$Y=11{\cdot}50x-26{\cdot}16$	432·1	512·3	661·9
Dimefox	$Y=5{\cdot}75x-14{\cdot}35$	1,652·0	2,317·0	3,864·0
Toxaphene	$Y=10x-31{\cdot}5$	3,673·0	4,467·0	5,998·0

which were limited to two deposits for each chemical, are tabulated alongside the average deposits which might be encountered on foliage after field applications.

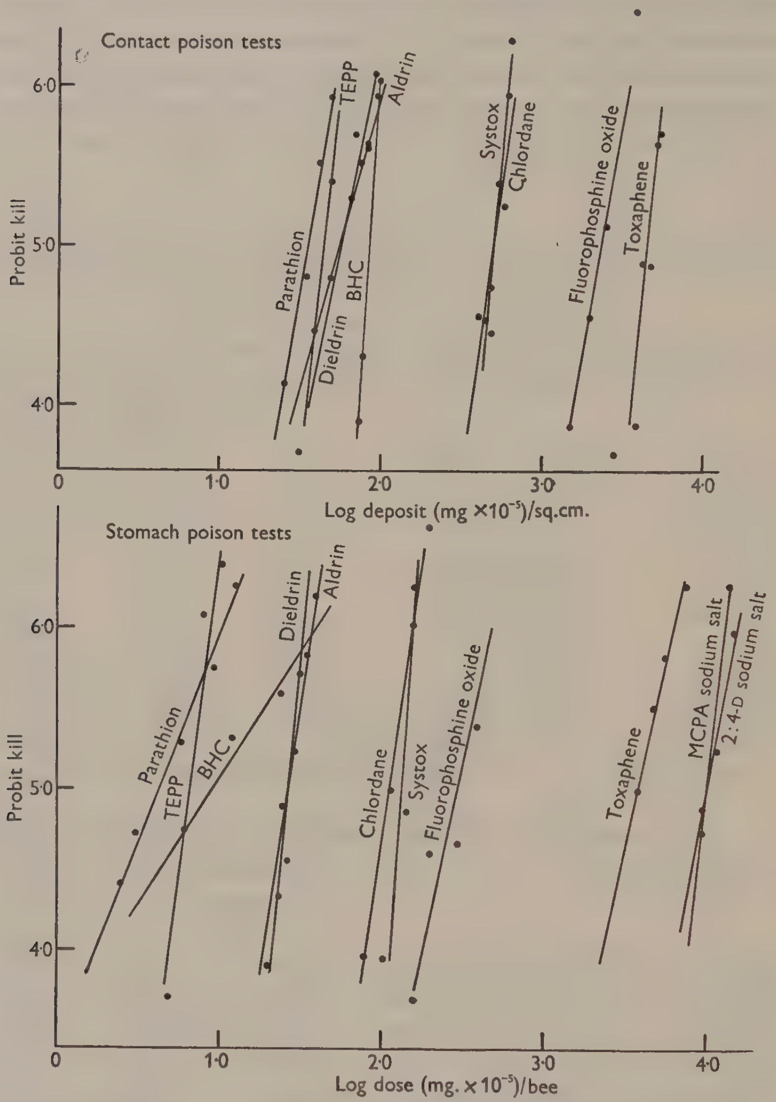

Fig. 2. Graphs showing relative toxicities of the various chemicals as stomach and contact poisons to worker honey-bees.

The salts of the chloro-phenoxyacetic acids had no appreciable contact effect.

In the stomach and contact poison tests the chemicals showed the same order of effectiveness, but as residual films and fumigants dieldrin, aldrin and γ-BHC were the most toxic. Toxaphene showed a very low toxicity to bees.

Table 5. *Toxic effect of maintaining bees for 1 hr. in contact with residual films of chemicals*

Material	Percentage kill in 24 hr.	Dry film (mg./sq.cm.)	Field application (average dose) (mg./sq.cm.)	Oz./acre
Dieldrin	90	0·00009	0·0014	2
	10	0·00004		
Aldrin	75	0·00009	0·0014	2
	0	0·00004		
γ-BHC	100	0·000280	0·0028	4
	0	0·000074		
Parathion	90	0·00054	0·0014	2
	10	0·00018		
Chlordane	100	0·00340	0·0112	16
	12	0·00090		
Systox	50	0·01	—	—
	22	0·0068		
TEPP	8	0·00022	0·0056	8
Toxaphene	9	0·11000	0·0168	24
	0	0·04000		
Dimefox	0	0·054	—	—

Table 6. *Toxic effect of vapours from residual films. Exposure period* 1 *hr.*

Material	Percentage kill in 24 hr.	Dry film (mg./sq.cm.)
Dieldrin	100	0·000280
	0	0·000074
γ-BHC	100	0·00044
	0	0·00028
Aldrin	100	0·000740
	0	0·000074
Parathion	100	0·0050
	0	0·0028
Chlordane	100	0·00370
	0	0·00037
Toxaphene	0	0·070
TEPP	0	0·0055
Systox	0	0·0185
Dimefox	0	0·0740

Discussion

In spite of the considerable amount of data published on honey-bee toxicology, little of it can be used for comparative purposes, as with the exception of stomach poison tests, few dosage/response relationships have been established.

The available figures, quoted either as published or calculated from the relevant data for stomach poison tests, are compared with the present results in Table 7.

The degree of agreement is considered to be satisfactory and the only exception, TEPP, is not expected to give consistent results owing to its rapid rate of hydrolysis in aqueous solution.

The field experiments of Weaver (1951) and Knowlton, Nye, Lieberman, Todd & Bohart (1950) tend to confirm the present results, particularly regarding the low toxicity of toxaphene.

TABLE 7. *Comparison between present data on stomach toxicity with that obtained by other workers*

Chemical	LD_{50} (μg.) Present data (24 hr.)	LD_{50} (μg.) Published data (72 hr.)	Source	Agreement
Parathion	0·04	0·07	Eckert (1949)	Good
		0·1	Hafliger (1950)	Satisfactory
		0·09 (24 hr.)	Salkeld (1951)	Satisfactory
TEPP	0·065	0·75	Eckert (1949)	Poor
Aldrin	0·22	0·25	Eckert (1949)	Good
Chlordane	1·1	1·21	Eckert (1949)	Good
		1·4	Weaver (1950)	Good
Toxaphene	39·8	22·0	Eckert (1949)	Satisfactory
		31·9	Weaver (1950)	Good

The experimental data contained in Tables 3–6 may be used as a basis for estimating the effect on the honey-bee, of the use of the various chemicals in the field, but taken by themselves they only form a guide, since a large number of other factors may influence toxicity. The most important are, so far as is known, the formulation of the chemical; its properties as a repellent; the time and method of application; the nature of the crop treated and the intensity of bee activity. The influence of some of these factors has been discussed by Eckert (1949), Hafliger (1949) and Hocking (1950). The theoretical considerations relating to the response of the honey-bee to repellent chemicals have been considered by Glynne Jones (1952); the penetration of dusts into the respiratory system by Connell & Glynne Jones (1953); and the effect of dusts on the bee's cuticular waterproofing mechanism by Glynne Jones (1954).

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THE PRODUCTION OF ANTIBIOTICS IN SOIL

I. PRODUCTION OF GLIOTOXIN BY *TRICHODERMA VIRIDE*

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Conditions affecting the production of gliotoxin by a strain of *Trichoderma viride*, known to produce this antibiotic in synthetic culture media, were studied in two types of soil, a highly acid, sandy podsol from Wareham Heath and a less acid garden soil. High yields of an antibiotic substance, which results from bioassays showed to be similar to gliotoxin, were obtained from both inoculated soils when autoclaved and supplemented with organic material. The autoclaved soils behaved differently when unsupplemented; Wareham soil supported production of the antibiotic but little or none was produced in the garden soil. No antibiotic activity could be demonstrated in soil which had not been inoculated with *T. viride*.

Acidification of unsupplemented garden soil by addition of sulphuric acid had a favourable effect on production of the antibiotic, but raising the pH of Wareham soil by addition of calcium hydroxide also increased the yield. These effects, therefore, cannot be due simply to the change in pH of the soil.

The beneficial effect of autoclaving the soil on production of the antibiotic assumed to be gliotoxin was analysed and separated into three distinct effects, elimination of the microflora, increase in availability of nitrogen compounds and increase in available carbon compounds. The last effect was considered to be of greatest significance.

The antibiotic was produced in normal Wareham soil if supplemented with additional carbon compounds, but not in garden soil unless this had also been acidified before inoculation. A chromatographic method of bioassay used in the later work gave more substantial evidence that the antibiotic produced in the soil was, in fact, gliotoxin.

INTRODUCTION

The first suggestions that antibiotics might be produced in soil and play a part in the stabilization of the micropopulation arose from studies of antagonism between soil-borne plant pathogens and soil saprophytes. Disease incidence could sometimes be reduced by inoculation of the infected soil with saprophytic organisms known to be antagonistic to the pathogen in synthetic media (Sanford & Broadfoot, 1931; Garrett, 1934; Subramanian, 1946; Slagg & Fellows, 1947). In recent years this antagonism has frequently been attributed to production of an antibiotic substance by the saprophyte (Brian, 1949; Garrett, 1950; Grossbard, 1952; Celino & Gottlieb, 1952).

The importance of *Trichoderma viride*, a constituent of the microflora of many soils, as such an antagonist has been stressed by many workers. In a study of control of damping-off of *Citrus* seedlings caused by *Rhizoctonia solani*, Weindling (1932, 1934) and Weindling & Fawcett (1936) found that if infected soil were inoculated with spores of *Trichoderma viride* good control of the disease was obtained. Better control was obtained in soils of low pH (less than 5·5) than in soils of high pH. Evidence was presented, based on *in vitro* experiments, that this was due to the production of a 'lethal principle' by the saprophyte; this was later isolated in crystalline form from cultures of the fungus on liquid media and named gliotoxin (Weindling & Emerson, 1936; Weindling, 1941).

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Timonin (1941) also suggested that *T. viride* played a part in disease resistance from a study of susceptibility of different varieties of flax to wilt caused by *Fusarium lini*. He found that *Trichoderma viride* was a prominent member of the rhizosphere of the resistant variety Bison. In soils in which *T. viride* was abundant, i.e. soils of low pH, it was also shown by Rishbeth (1949) that disease of *Pinus* caused by infection of the roots by *Fomes annosus* was not severe.

More recently attention has been given to obtaining direct evidence of production of antibiotics in soil, but this has not yet been achieved although it has been shown that soil, when suitably treated, can support growth and antibiotic production by many soil organisms.

Grossbard (1952) has demonstrated the production of an antibiotic in autoclaved and also in partially sterilized and supplemented soil inoculated with *Penicillium patulum*. It has also been shown by Hessayon (1951) that an antibiotic substance could be produced in unsupplemented but autoclaved soil inoculated with *Trichothecium roseum* (an organism which produces the antibiotic trichothecin). Similarly, Gregory, Allen, Riker & Peterson (1952) have demonstrated the production of antibiotic activity in unsterile soil which had been supplemented with soybean meal, glucose and corn-steep liquor and inoculated with *Penicillium patulum*. In these cases it was assumed, but not conclusively demonstrated, that the antibiotic found in the soil was identical with that produced in synthetic media. Gottlieb & Siminoff (1952), however, demonstrated the production of an antibiotic by *Streptomyces venezuelae* in sterilized but unsupplemented soil, and identified it as chloromycetin by its chemical reactions and, with the aid of paper chromatography, by its similarity in R_F value with that of pure chloromycetin. This work has been concerned mainly with sterile soil, that is soil which has been subjected to and therefore modified by heat treatment, or as in the experiments of Gregory *et al.* with a heavily supplemented soil. Moreover, in none of these cases was the organism studied a common soil saprophyte.

A study has therefore been made of the conditions under which *Trichoderma viride*, a common and widely distributed soil fungus, will produce gliotoxin in soil.

MATERIALS AND METHODS

Culture. The organism used throughout was *T. viride* Pers. ex Fries (our strain 211). This strain has been shown to produce high yields of gliotoxin in liquid culture (Brian & Hemming, 1945).

Soils. Two contrasting types of soils were used, a highly acid (pH 4·5–5·2), sandy podsol from Wareham Heath taken from the humus-rich A_1 horizon and a less acid garden loam (pH 5·6–6·5) from Hertfordshire. These are referred to as Wareham and garden soils respectively. The soil was sieved, brought to a constant water content (20%) and dispensed in 60 g. (dry weight) portions in milk bottles. In some experiments the soil was autoclaved at 15 lb/in.^{2} for 45 min. Soil not treated in this way is referred to throughout as 'normal' soil to distinguish it from 'autoclaved' soil.

Inoculation. A suspension of *T. viride* spores in distilled water was prepared and standardized with the aid of a haemacytometer, to 2×10^6 spores/ml. Each bottle of soil (60 g.) was inoculated with 2·5 ml. of this suspension.

Extraction of antibiotic from soil. After incubation of the soil at 25° C. for the required period (usually 7 days) the water content was estimated and 100 g. (dry weight) of soil was continuously extracted with ether in a Soxhlet apparatus. The extract was evaporated to dryness and the residue dissolved in 2 ml. ethanol and 18 ml. citric acid (M/1000) and adjusted to a pH of 3·5 at which gliotoxin would be expected to be fairly stable.

Assay. Method 1. The extract was assayed for antifungal activity by the serial dilution spore germination method with *Botrytis allii* as the test organism (Brian & Hemming, 1945) and for antibacterial activity by the cylinder plate method using *Bacillus subtilis* as the test organism. In each case the inhibitory activity was compared with that produced by standard solutions of gliotoxin and the concentration present in the soil assessed. In earlier experiments no real proof was available that the antibiotic produced in the soil was gliotoxin, although the results given by the bioassay with two test organisms supported the view that it was mainly gliotoxin. This assumption was later shown to be substantially correct by results obtained from the second, more specific, method of assay described below.

Method 2. The previous method of assay could detect 1·0 μg. gliotoxin/g. soil and this was found to be adequate for demonstrating production of the relatively large quantities of gliotoxin found in autoclaved soil, but when studying production in normal soil a more sensitive method of assay was required. A bioassay based on paper chromatography was therefore devised by which smaller quantities of the antibiotic could be detected.

The soil extracts, or solutions of pure gliotoxin (in ethanol:citric acid (M/1000), 1:1) used as standards, were chromatographed on filter-paper strips (Whatman no. 1). A mixture of ethanol and acetic acid was used as the solvent (ethanol/0·1 N acetic acid/water in the ratio 2·5:2·0:5·5). After developing for 6 hr. at 25° C. the strips were dried and placed on a large agar plate seeded with *B. subtilis*. After incubation at 30° C. for 12 hr. the strips were removed and the distance moved by the active substance was indicated by the position of a clear zone of inhibition, and from this measurement and that of the distance moved by the solvent front the R_F values were calculated. From a comparison of the diameter of the inhibition zone with those produced by standard solutions of gliotoxin it was possible to estimate approximately the amount of gliotoxin present in the soil extract. By this method it was possible to detect 0·15 μg. gliotoxin/g. soil. Similarity of R_F values produced by the active material in the soil extract and the standard solutions afforded additional evidence that the antibiotic produced was, in fact, gliotoxin.

EXPERIMENTAL RESULTS

Addition of organic supplements to autoclaved soils

The following plant materials, dried and ground, were mixed with Wareham and garden soils (5% on a dry-weight basis): bracken fronds, *Deschampsia* foliage, Scots Pine needles, clover foliage and wheatmeal. The first three of these were normal constituents of the surface litter on Wareham Heath. After addition of the supplements the soil was dispensed in bottles and autoclaved. The soil was then inoculated with *Trichoderma viride*, incubated for 7 days at 25° C., extracted and assayed for gliotoxin. Parallel uninoculated controls were also set up. No gliotoxin was produced in uninoculated soil; data for inoculated soils are presented in Table 1.

TABLE 1. *Effect of addition of organic supplements to autoclaved soils on production of gliotoxin by* Trichoderma viride

	Gliotoxin concentration (μg./g. dry soil)	
Supplement	(*a*)	(*b*)
Wareham soil		
None	5·0	2·8
Bracken	10·0	4·2
Pine needles	6·0	4·2
Deschampsia	8·0	3·8
Clover	20·0	10·0
Wheatmeal	320·0	40·0
Garden soil		
None	2·0	0
Bracken	6·0	4·2
Pine needles	5·0	2·4
Wheatmeal	20·0	40·0

(*a*) From antifungal assay; (*b*) from antibacterial assay.

In Wareham soil particularly, the estimated gliotoxin content was consistently higher if calculated from the antifungal assay than from the antibacterial assay. This discrepancy can also be seen in subsequent data, particularly under acid soil conditions. Gliotoxin-producing strains of *T. viride* produce also small quantities of viridin (Brian, personal communication), a highly antifungal substance with no antibacterial properties. It is likely that in those experiments where pH conditions are suitable for accumulation of viridin, production of traces of viridin has introduced an error into the gliotoxin determinations by antifungal assay. For that reason estimations of gliotoxin concentrations based on antibacterial assay should be regarded as more reliable.

Wareham soil. Some gliotoxin was produced in unsupplemented autoclaved soil and in this case growth of the fungus was confined to isolated patches on the surface of the soil probably in the region of naturally occurring plant debris. All supplements increased production but by far the most effective were clover and wheatmeal. These results roughly corresponded to the extent of visible growth on the soil surface.

Garden soil. In this soil no gliotoxin was produced without supplement. All supplements stimulated gliotoxin production; with wheatmeal the yields of gliotoxin were as great as in Wareham soil. Here also visible growth was greatest on the treatments where gliotoxin production was greatest.

pH *of soils*

Unsupplemented garden soil (pH 5·6) was adjusted to pH's 4·2 and 3·6 with dilute sulphuric acid. After autoclaving, the soils were inoculated with *T. viride*, incubated for 7 days at 25° C. and the gliotoxin content assayed as before. The results presented in Table 2 show that acidification tended to increase gliotoxin production. The production of a little gliotoxin in autoclaved but otherwise untreated Garden soil in this experiment was unusual; in most other experiments, including many not reported here, no gliotoxin was produced under such conditions, as shown in Table 1. The effect of pH adjustment was not great. No gliotoxin was produced in uninoculated controls.

Wareham soil (pH 4·8) was adjusted to pH's 7·1, 7·8 and 8·3 with calcium hydroxide before autoclaving and inoculation in the usual manner. Each increase in pH in this range increased the yield of gliotoxin (Table 2). This result was unexpected in view of the known instability of gliotoxin near neutrality (Jefferys, 1952). The possibility that the increase was due to an effect of calcium ions, rather than to the change in pH, was considered, but from experiments in which calcium in the form of calcium chloride was added to the soil results were inconclusive. No gliotoxin was produced in uninoculated controls.

Analysis of the effect of autoclaving soil

On several occasions it was found that while gliotoxin production could be detected in autoclaved but unsupplemented soil, none could be detected in normal soil which had received no supplement or heat treatment. The effects of autoclaving on soils are complex. Those likely to be of greatest importance in this connexion were considered to be: (*a*) elimination or reduction in numbers of the microflora and microfauna; (*b*) increase in available nitrogen; (*c*) increase of available energy-yielding carbon compounds as a result of breakdown of organic matter. Some experiments to determine the relative importance of these effects were therefore carried out.

TABLE 2. *Effect of acidification of garden soil with sulphuric acid and of addition of calcium hydroxide to Wareham soil, before autoclaving, on production of gliotoxin by* Trichoderma viride

	pH of soil			Gliotoxin concentration (μg./g. dry soil)	
Treatment	Before autoclaving	After autoclaving	After 7 days' incubation	(*a*)	(*b*)
Wareham soil					
None	4·8	4·0	3·9	5·0	1·8
0·4 % $Ca(OH)_2$ added	7·1	5·4	5·6	5·0	1·9
0·6 % $Ca(OH)_2$ added	7·8	6·5	6·3	20·0	3·6
0·8 % $Ca(OH)_2$ added	8·3	7·4	6·9	30·0	8·4
Garden soil					
None	5·6	5·4	6·3	2·0	1·5
H_2SO_4 added	4·2	4·2	4·7	2·4	0
H_2SO_4 added	3·6	3·6	3·7	11·0	3·4

(*a*) From antifungal assay; (*b*) from antibacterial assay.

TABLE 3. *Effect of re-infection of autoclaved Wareham soil with the normal soil microflora on production of gliotoxin by* Trichoderma viride

	Gliotoxin concentration (μg./g. dry soil)	
Soil treatment	(*a*)	(*b*)
Untreated (normal)	0	0
Autoclaved	10·0	6·6
Autoclaved and re-infected	2·4	2·4
Autoclaved and supplemented with wheatmeal	320·0	16·0
Autoclaved, supplemented, and re-infected	320·0	15·2

(*a*) From antifungal assay; (*b*) from antibacterial assay.

Removal of microflora. A mixed soil microflora was re-introduced into autoclaved Wareham soil by incorporating 1·0 g. normal soil into each 60 g. of autoclaved soil; supplemented (5 % wheatmeal) and unsupplemented soils were used. Gliotoxin yields after inoculation with *T. viride* and incubation at 25° C. for 7 days are shown in Table 3. Re-infection with a normal soil microflora reduced but did not prevent gliotoxin production in unsupplemented soil, but had little effect in supplemented soil. Further evidence that the beneficial effect of autoclaving is not mainly a consequence of its sterilizing effect is afforded by the data in Table 4; 5 min. steaming eliminated the bacterial and fungal microflora, but a minimum of 25 min. steaming was necessary to make the soil a suitable medium for gliotoxin production.

Available nitrogen. Steam was blown through Wareham soil in an adapted domestic steamer. The soil temperature reached 100° C. in 2 min. and samples were removed every following 5 min. up to 45 min. Plate counts of fungi and bacteria were made on a portion of each sample, amino-N, nitrate-N and ammonia-N content determined, and the remainder of each sample inoculated with *T. viride*, incubated at 25° C. for 7 days and the gliotoxin present determined by bioassay. Results are presented in Table 4. As has already been noted, gliotoxin production could not be detected in soil steamed for less than 25 min., a treatment considerably in excess of that needed to sterilize the soil. All three forms of nitrogen increased as a result of steaming. Even after steaming, the levels of nitrate-N and amino-N were very low, and it was not considered likely that the increase in these forms of nitrogen could have affected gliotoxin production. The increase in ammonia-N was more substantial, about 12·5 μg./g. soil being produced as a result of 45 min. steaming. However, in subsequent experiments addition of ammonium tartrate, equivalent to this increase in ammonia-N, had no effect on gliotoxin production in either normal or autoclaved Wareham soil.

TABLE 4. *Effect of steaming Wareham soil on numbers of fungi and bacteria, nitrogen content of the soil, and on gliotoxin production by* Trichoderma viride

Duration of steaming (min.)	Plate counts (thousands/g. dry soil)		Nitrogen content (μg./g. dry soil)			Gliotoxin concentration (μg./g. dry soil)	
	Fungi	Bacteria	Amino-N	Nitrate-N	Ammonia-N	(*a*)	(*b*)
—	60	89	—	0·36	14·10	—	—
5	< 1	< 1	0·97	0·63	16·45	—	—
10	< 1	< 1	0·74	0·64	18·29	—	—
15	< 1	< 1	1·16	0·91	24·95	—	—
20	< 1	< 1	0·99	1·41	23·32	—	—
25	< 1	< 1	—	—	—	1·2	1·0
30	< 1	< 1	1·15	2·7	26·64	2·4	1·2
45	< 1	< 1	1·19	3·3	26·56	5·0	2·0

(*a*) From antifungal assay; (*b*) from antibacterial assay.

Available energy-yielding carbon compounds. Preliminary experiments showed some production of gliotoxin in normal Wareham soil if supplemented with wheatmeal or dried clover, but the amount produced was close to the limits of the technique of the direct assay method used. The more sensitive method based on paper chromatography, described earlier in this paper, was devised to overcome these limitations and used in all work described below.

The results of a number of experiments are summarized in Table 5. No gliotoxin was produced in uninoculated controls. In normal inoculated Wareham soil addition of clover stimulated production of gliotoxin and a supplement as little as 1 % was effective, although the quantity produced was considerably less than that produced in autoclaved soil. In these supplemented soils *Zygorhynchus moelleri* grew vigorously on the surface but in inoculated treatments growth of *Trichoderma viride* could also be seen. No gliotoxin was produced in garden soil even though supplemented with dried clover unless the soil was previously acidified, although growth of *T. viride* was equally vigorous in unacidified soil. The R_F values for the antibiotic produced in soil by *T. viride* are very close to that of pure gliotoxin; the assumption made throughout this paper that gliotoxin is the main antibiotic produced in soil by the strain of *T. viride* used is justified by this evidence.

TABLE 5. *Effect of supplementing normal Wareham and garden soils on gliotoxin production by* Trichoderma viride, *based on data obtained from a paper chromatogram assay*

Treatment	R_F of active material	Zone diameter (mm.)	Gliotoxin concentration (μg./g. dry soil)
Wareham soil (Exp. 1)			
Normal soil + 5 % clover	0·63	18	0·15
Autoclaved soil + 5 % clover	0·62	32	5·0
Gliotoxin (100 μg./ml.)	0·62	33	—
Wareham soil (Exp. 2)			
Normal soil	—	0	0
Normal soil + 1 % clover	0·65	*	*
Normal soil + 2 % clover	0·64	*	*
Normal soil + 5 % clover	0·62	*	*
Gliotoxin (25 μg./ml.)	0·66	26	—
Garden soil			
Normal soil (pH 6·5)	—	0	0
Normal soil + 5 % clover	—	0	0
Acidified soil (pH 3·6) + 5 % clover	0·68	22	1·2
Gliotoxin (25 μg./ml.)	0·69	21	—

* An obvious zone of inhibition produced in assay, but owing to 'tailing' it was not possible to make satisfactory measurements.

DISCUSSION

These experiments have shown that a variety of soil conditions affect the production of gliotoxin by *T. viride*; nevertheless, it is now possible in a preliminary way to assess the importance of some of them.

In none of the experiments has it been found possible to detect gliotoxin in either of the types of soil inoculated with *T. viride* with no further treatment. If Wareham soil is autoclaved before inoculation it becomes suitable for production of small quantities of gliotoxin but garden soil similarly treated is less suitable, gliotoxin being produced only rarely. Both soils, if supplemented with certain plant residues before autoclaving, become very favourable media for gliotoxin production. Of the various supplements added wheatmeal and dried clover were most effective, and these were the supplements which might be expected to contain the largest amount of available carbohydrate.

Gliotoxin production might well be expected to be greater in acid soils, in view of the greater stability of gliotoxin at low pH. Such an expectation is supported by the favourable effect of acidification of garden soil (Tables 2 and 5), and the general superiority of autoclaved but unsupplemented Wareham soil over garden soil. On the other hand, a low pH cannot be regarded as an essential prerequisite for gliotoxin production since, after raising the pH of autoclaved Wareham soil to near neutrality by addition of calcium hydroxide, marked increases in gliotoxin production were observed.

The beneficial effect of autoclaving is complex. The results presented show that this is to some extent due to removal of competing micro-organisms but not entirely. Re-infection of autoclaved soil (Table 3) reduced but did not prevent gliotoxin production and had no effect on gliotoxin yield in soils supplemented with wheatmeal before autoclaving. In other experiments (Table 4) it was shown that the beneficial effect of heating only became marked after a period of heat treatment considerably in excess of that required to kill the microflora. The effect of autoclaving on available nitrogen could not be related to enhanced gliotoxin production (Table 4). A further possibility to be considered was that autoclaving might increase the amount of available energy-yielding carbon compounds in the soil and so increase gliotoxin production. This in fact appears to be a likely explanation; it was not directly proved, but addition of small quantities of dried clover, relatively high in carbon content, to otherwise untreated Wareham soil did stimulate gliotoxin production. Similar results were obtained with garden soil when acidified and supplemented with dried clover.

Though it has been possible to demonstrate production of an antibiotic in normal Wareham soil when supplemented with 1 % dried clover, and to identify this with some confidence as gliotoxin by chromatographic methods, it must be admitted that addition of 1 % dried clover is a fairly considerable soil amendment. Furthermore, under these conditions, or even with heavier supplements of clover, when expressed in terms of dry weight of soil the concentrations of gliotoxin found in the soil are low. Nevertheless, it appears likely that the fungus will grow and produce gliotoxin primarily in the vicinity of patches of organic supplement, and that in those local micro-environments much greater concentrations of gliotoxin will occur. Further attention is being devoted to a study of this aspect of gliotoxin production in soil.

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BIOLOGICAL TECHNIQUES FOR THE EVALUATION OF FUNGICIDES

III. THE EVOLUTION OF A TECHNIQUE FOR THE EVALUATION OF SOIL FUNGICIDES FOR THE CONTROL OF CLUB-ROOT DISEASE OF BRASSICAE

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The effectiveness of fungicides for the control of *Plasmodiophora brassicae* Woron. in pot tests under controlled conditions is influenced by spore load, duration of storage of spores prior to inoculation, time of year and temperature. Many fungicides are less effective in soil which has been heavily treated with calcium hydroxide than in unlimed soil or in soil treated with calcium carbonate. In general, fungicides are less efficient when very heavy attacks of the disease occur. If experiments are made under comparable conditions consistent conclusions can be obtained.

A schedule of testing is recommended, and it is suggested that fungicides found to be satisfactory in a preliminary test should be subjected to a detailed test. The proposed method of evaluation of a fungicide is to compare its efficacy with that of a standard treatment under comparable conditions. For comparative purposes the efficiency of a fungicide is measured by the percentage number of diseased plants occurring in treated soil and also by the disease index.

Introduction

Club root may be regarded as the most serious disease of Brassicae occurring in the temperate zone. This is largely due to the survival of the causal organism, *Plasmodiophora brassicae* Woron., in the soil for many years and the difficulty of eradicating it by economic methods. On contaminated land it is still necessary to abandon the growing of susceptible crops for long periods or to make continual use of fungicides and other soil treatments. Since the incidence of the disease is greatly affected by a number of environmental factors it is not surprising that conflicting views exist regarding the efficacy of many control measures, assessments having hitherto been made mainly under uncontrolled conditions.

In the present work the aim has been to develop a pot method to enable soil disinfectants and treatments to be tested for their efficacy under controlled conditions. Some fungicides employed for soil treatment induce alkalinity, but results of a study of the epidemiology of the disease (Colhoun, 1953*a*) make it possible to separate fungitoxic properties from effects due to soil alkalinity. It is therefore now possible to attempt to evaluate the efficacy of fungicides by means of a pot test made under controlled conditions whereby the severity of the disease can be predetermined.

Biological techniques have already been evolved for the evaluation of seed disinfectants for the control of diseases of oats and flax (Muskett, 1938; Muskett & Colhoun, 1942). Bremer, Wehnelt & Brandenburg (1937) contributed towards the development of a pot technique for evaluating fungicides for the control of *P. brassicae* and studied the effects of spore load on their efficacy, but little attention was paid to soil reaction. It may be suggested that the considerable variation they reported in the efficacy of substances such as lime in different experiments may be due to fluctuations in uncontrolled factors.

MATERIALS AND METHODS

The methods employed were similar to those already described (Colhoun, 1953*a*).

Four soils were employed: (*a*) Stranmillis soil, a medium loam of pH 6·2; (*b*) Narrow Water soil, a medium loam of pH 6·3; (*c*) Newtownards soil, a light loam of pH 6·2; and (*d*) Stormont soil, a light loam of pH 4·8. Both the Stranmillis and Narrow Water soils had borne crops very seriously attacked by *P. brassicae* for a number of years while the Newtownards soil had produced crops which were only slightly affected. The Stormont soil was known to be free from the parasite and was used in all experiments where artificial contamination was employed. The inoculum used to contaminate the soil was obtained from different sources in Co. Down and was from diseased cabbage roots except in one experiment where material from a cauliflower crop was used. No inoculum was added to the Stranmillis, Narrow Water or Newtownards soils. Spore load is always expressed as the number of spores per g. of oven-dried soil.

During the work laboratory chemicals, proprietary fungicides and experimental formulations were employed. The materials used, the active ingredients of proprietary fungicides and the form in which applied to the soil were as shown on p. 292.

The requisite amount of a fungicide in powder form was thoroughly incorporated with the air-dried soil, the soil reaction having been first adjusted by addition of $Ca(OH)_2$ or $CaCO_3$ if necessary. The amounts of $Ca(OH)_2$ and $CaCO_3$ added to each bulk corresponding to 3000 g. of oven-dried soil were 14 and 33 g. respectively. If the soil was uncontaminated with *P. brassicae* it was then inoculated, the appropriate spore load being applied. Water was then added to the naturally or artificially contaminated soil to provide a standard moisture content of 70 % of the maximum water-holding capacity. After the addition of the water the soil was again thoroughly mixed and placed in water-tight tins which served as pots. When a fungicide in solution was employed, the appropriate volume at the desired concentration was thoroughly mixed with the soil immediately before the moisture content was adjusted. All soil was mixed by hand at each stage of the work. The rate of application of fungicides is expressed later as the amount applied to a bulk of soil equivalent to 3000 g. of oven-dried soil.

Throughout the work cabbage seedlings (variety Enfield Market), raised in sterilized compost, were transplanted into soils which were untreated or treated with a fungicide under test. Twenty seedlings were planted in each pot. In limed soil a delay of 5 days occurred between treatment and transplanting, but there was little delay when unlimed soils were used. An exception was made in the case of treatment with formalin where a delay of 14 days was allowed. The soil was maintained throughout each experiment at a relatively constant moisture content equal to 70 % of the maximum water-holding capacity by weighing the pots on alternate days and replacing the water lost. Different temperature ranges were employed in different experiments. In 1935 and 1936 rather low temperatures were used, but in 1951 and 1952 the pots were maintained throughout each experiment in a heated greenhouse at high temperatures which had been shown to favour the most severe attacks of the disease in acid or alkaline soils (Colhoun, 1953 *a*, *b*). At the conclusion of most experiments a value of 10 was given to pots in which the plants had grown best, the others being assessed at between 10 and 0 according to the growth made.

	Active ingredient	Form in which used
Laboratory chemicals		
Mercuric chloride	—	Solution
Copper sulphate	—	Solution
Potassium permanganate	—	Solution
Boric acid	—	Solution
Carbolic acid	—	Solution
Sodium carbonate (crystalline washing soda)	—	Solution
Formalin	Approx. 40 % formaldehyde	Solution
Calcium hydroxide	—	Powder
Calcium carbonate	—	Powder
Proprietary fungicides		
Abavit S (British Schering) (containing 2 % Hg)	Phenyl mercury urea derivative	Solution
Aretan (Bayer) (containing 3 % Hg)	Methoxyethyl mercury chloride	Solution
Uspulun (Bayer) (containing 18 % Hg)	Chlorphenyl mercury derivative	Solution
C.R.C. (Bayer)	4 % mercurous chloride	Powder
Brassisan (Bayer)	20 % trichlorodinitrobenzene	Powder
Orthocide (SR 406) (Esso)	50 % N-trichloromethylthiotetrahydrophthalimide	Suspension
Experimental materials		
Sch. 1179 (Bayer)	10 % trichlorodinitrobenzene	Solution
Sch. 1150 (Bayer)	20 % trichlorodinitrobenzene	Powder
DA 19101 (Bayer)	20 % dinitrothiocyanobenzene	Powder
DA 1604 (Bayer)	20 % hexachlorobenzene	Powder
10101 (Bayer)	20 % methyldiethyl dithiocarbamate	Powder

RESULTS

Experiments with naturally contaminated soils

Preliminary experiments made during 1935 in acid soil at a mean air temperature of about 15° C. showed that the incidence of the disease was greatly reduced by treatment with mercuric chloride, carbolic acid, formalin, Uspulun or Aretan, although carbolic acid was phytotoxic. Solutions of copper sulphate, 0·25–2·0 %; potassium permanganate, 0·5–2·0 %, and boric acid, 0·001–0·002 %, effected little control. When alkaline soils were used, little or no disease developed even in soil not treated with a fungicide.

Experiment 1. This experiment was made with Narrow Water soil from 24 June to 17 August 1936 in an open-air verandah with a mean air temperature of 15·2° C. (range 9–22° C.). Ten treatments were employed, in addition to the untreated soil which served as a control, two pots constituting a unit for each treatment. The results obtained for the mean percentage number of diseased plants for each treatment are stated in Table 1.

TABLE 1. *Efficacy of soil treatments in different soils at low or moderate temperatures*, 1936

(Soils naturally contaminated.)

	Mean % no. of diseased plants					
	Narrow Water soil		Stranmillis soil	Newtownards soil	Soil pH at planting	
Treatment (per 3000 g. oven-dried soil)	Exp. 1, 15·2° C.	Exp. 2, 17·9° C.	Exp. 3, 17·7° C.	Exp. 4, 17·7° C.	Stranmillis soil	Newtownards soil
Untreated	52·8	100·0	96·2	54·2	6·2	6·2
$HgCl_2$, 0·1 %, 200 ml.	0	18·2	0	0	6·2	6·1
$HgCl_2$, 0·2 %, 200 ml.	0	0	0	0	6·4	6·1
Aretan, 2 %, 200 ml.	—	—	2·1	1·5	7·3	7·3
Aretan, 4 %, 200 ml.	0	0	0	0	7·6	7·4
Uspulun, 1 %, 200 ml.	—	—	0	0	7·0	6·6
Uspulun, 2 %, 200 ml.	0	0	0	0	6·9	6·6
Washing soda, 10 %, 200 ml.	—	—	17·5	—	8·1	—
1179, 1 %, 200 ml.	0	97·5	68·4	—	6·6	—
1179, 2 %, 200 ml.	0	38·1	11·1	3·6	6·6	6·3
Brassisan, 20 g.	—	—	2·0	—	6·3	—
Brassisan, 40 g.	0	0	1·1	0	6·1	6·1
Brassisan, 80 g.	0	0	—	—	—	—
1150, 20 g.	—	—	3·2	—	7·0	—
1150, 40 g.	0	0	0	0	7·6	7·5
1150, 80 g.	2·6	0	—	—	—	—

Experiment 2. The previous experiment was repeated from 13 September to 10 October 1936 employing a mean air temperature of 17·9° C. (range 5–35° C.), and the results obtained are also shown in Table 1.

Experiment 3. This experiment was made with Stranmillis soil from 31 August to 29 September 1936 in a greenhouse with a mean air temperature of 17·7° C. (range 8–34° C.). Washing soda was employed as well as the fungicides tested in Exp. 2. A unit consisted of five pots for each treatment and of ten pots for the untreated soil. The soil pH values at planting time, together with the mean percentage number of diseased plants for each treatment and the control, are presented in Table 1.

Experiment 4. Most of the treatments used in Exp. 3 were employed in this experiment made with Newtownards soil from 5 September to 1 October 1936 in a greenhouse with a mean temperature of 17·7° C. (range 5–34° C.). Five pots constituted a unit for each treatment. The results are shown in Table 1.

Comparison of results

Exps. 1 and 2, made with Narrow Water soil, differed in that a higher temperature range was employed in Exp. 2. At the higher temperatures more severe attacks occurred and some fungicides were less effective.

In Exps. 2–4, made with different soils under comparable conditions of soil moisture, temperature and alkalinity, one fungicide was more effective in the Newtownards soil which was believed, as a result of field observations, to be the least heavily contaminated. A less severe attack also occurred in the untreated pots with the less heavily contaminated soil.

Experiments with artificially contaminated soil

(*a*) *Efficacy of soil treatments at different levels of spore load employing fresh spores or spores stored for varying periods*

Experiment 5. This experiment was made from 6 July to 9 August 1951 in a heated greenhouse with a mean air temperature of 25·1° C. (range 12–43° C.), this temperature being much higher than that employed in previous experiments. Eighteen treatments were tested in acid soil as well as in soil treated with $Ca(OH)_2$ or $CaCO_3$. In the unlimed soil and in that treated with $Ca(OH)_2$ two spore loads, 10^3 and $2·5 \times 10^7$, were employed but in soil treated with $CaCO_3$ the spore loads used were 10^5 and $2·5 \times 10^7$. The spores utilized had been stored for 5 months after collection. Two pots constituted a unit for each treatment. The results obtained for the mean percentage number of diseased plants and disease index are presented in Table 2 in which are also stated the values for soil pH at the time of planting.

It is seen from Table 2 that fungicide 10101 at the two higher dosages killed all the plants in both limed and unlimed soil. Observations on plant growth were also made and these showed that even at the lowest dosage employed this fungicide inhibited growth in unlimed, but not in limed, soil. When used at the highest rate fungicide DA 19101 reduced growth in all soils and brought about considerable discoloration of the roots at all dosages. In general, the plants made better growth in limed as compared with unlimed soil.

Experiment 6. In this experiment the spores used for inoculating soil had been stored for 7 months, that is for 2 months longer than those employed in Exp. 5, but otherwise the conditions were similar. The experiment was in progress from 31 August to 7 October 1951 when the mean air temperature was 25·2° C. (range 16–41° C.). Each treatment was tested (*a*) in unlimed soil with spore loads of 10^3 and $2·5 \times 10^7$, (*b*) in soil treated with $Ca(OH)_2$ at spore loads of 10^5 and $2·5 \times 10^7$, and (*c*) in soil treated with $CaCO_3$ at a spore load of $2·5 \times 10^7$. Two pots constituted a unit for each treatment under each set of conditions. The results obtained are presented in Table 3.

None of the treatments seriously inhibited plant growth, but fungicide DA 19101 used at the highest rate caused discoloration of the roots.

It is seen from Table 3 that heavy attacks did not occur except at the high spore load in unlimed soil.

TABLE 2. *Effects of soil treatments and varying spore loads using spores stored for 5 months*, 1951

	Unlimed soil			Soil treated with $Ca(OH)_2$			Soil treated with $CaCO_3$		
		Mean % no. diseased plants and disease index*			Mean % no. diseased plants and disease index			Mean % no. diseased plants and disease index	
Treatment (per 3000 g. oven-dried soil)	Soil pH at planting	Spore load		Soil pH at planting	Spore load		Soil pH at planting	Spore load	
		$2·5 \times 10^7$	10^3		$2·5 \times 10^7$	10^3		$2·5 \times 10^7$	10^5
Untreated	4·8	100·0 (334)	72·5 (200)	7·1	97·4 (249)	5·0 (5)	7·4	11·4 (28)	0 (0)
$HgCl_2$, 0·1 %, 200 ml.	4·8	0 (0)	0 (0)	7·1	5·0 (5)	0 (0)	7·4	0 (0)	0 (0)
$HgCl_2$, 0·1 %, 400 ml.	4·7	0 (0)	0 (0)	7·3	0 (0)	0 (0)	7·4	0 (0)	0 (0)
$HgCl_2$, 0·2 %, 100 ml.	4·8	0 (0)	0 (0)	7·3	0 (0)	0 (0)	7·4	0 (0)	0 (0)
$HgCl_2$, 0·2 %, 200 ml.	4·7	0 (0)	0 (0)	7·2	2·5 (3)	0 (0)	7·4	0 (0)	0 (0)
Abavit S, 0·5 %, 200 ml.	5·1	100·0 (341)	15·0 (38)	7·3	90·0 (318)	7·5 (8)	7·4	22·5 (40)	0 (0)
Abavit S, 1·0 %, 200 ml.	5·5	90·0 (258)	0 (0)	7·4	79·4 (200)	0 (0)	7·5	5·0 (5)	0 (0)
C.R.C., 5 g.	4·8	0 (0)	0 (0)	7·2	0 (0)	0 (0)	7·5	0 (0)	0 (0)
C.R.C., 10 g.	4·8	0 (0)	0 (0)	7·2	0 (0)	0 (0)	7·5	0 (0)	0 (0)
C.R.C., 20 g.	4·8	0 (0)	0 (0)	7·2	0 (0)	0 (0)	7·4	0 (0)	0 (0)
DA 19101, 5 g.	4·9	0 (0)	15·0 (20)	7·2	15·0 (15)	0 (0)	7·5	0 (0)	0 (0)
DA 19101, 10 g.	4·8	0 (0)	0 (0)	7·3	0 (0)	0 (0)	7·5	0 (0)	0 (0)
DA 19101, 20 g.	4·8	0 (0)	0 (0)	7·2	0 (0)	0 (0)	7·5	0 (0)	0 (0)
DA 1604, 5 g.	4·8	100·0 (355)	17·5 (50)	7·2	97·5 (248)	25·0 (50)	7·4	23·4 (52)	2·5 (3)
DA 1604, 10 g.	4·8	97·5 (308)	40·0 (95)	7·1	100·0 (350)	17·5 (18)	7·3	12·8 (30)	2·5 (3)
DA 1604, 20 g.	4·8	97·4 (322)	22·5 (50)	7·1	95·0 (295)	15·0 (30)	7·4	0 (0)	0 (0)
10101, 5 g.	4·8	0 (0)	0 (0)	7·2	9·4 (9)	0 (0)	7·5	0 (0)	0 (0)
10101, 10 g.	4·8	All plants killed		7·3	All plants killed		7·5	All plants killed	
10101, 20 g.	4·8	All plants killed		7·2	All plants killed		7·5	All plants killed	

* The values for disease index are given in brackets.

Experiment 7. Spores stored for 2 months were used in this experiment which was made in a heated greenhouse with a mean air temperature of 24·7° C. (range 15–42° C.) from 14 April to 16 May 1952. All the treatments employed in Exp. 6 (except fungicide 10101), together with Aretan, washing soda and Orthocide at the concentrations stated in Table 4, were used. Each treatment was tested under the same soil conditions as in Exp. 6. Two pots constituted a unit for each treatment. The results obtained are stated in Table 4.

It is seen from Table 4 that heavy attacks occurred under all conditions and that many treatments were less effective in soil treated with $Ca(OH)_2$. The plants in limed soil developed better root systems and made better growth than those in unlimed soil. It was observed that Orthocide was phytotoxic at the higher concentrations, not only inhibiting growth but causing the development of scorched patches on the leaves.

It is of interest to note from Table 4 that in soil treated with $Ca(OH)_2$ the addition of washing soda increased the soil pH to 8·2, but at the higher spore load all the plants were seriously diseased.

TABLE 3. *Effects of soil treatments and varying spore loads using spores stored for 7 months,* 1951

	Unlimed soil			Soil treated with $Ca(OH)_2$			Soil treated with $CaCO_3$	
		Mean % no. diseased plants and disease index			Mean % no. diseased plants and disease index			Mean % no. diseased plants and disease index
Treatment (per 3000 g. oven-dried soil)	Soil pH at planting	Spore load		Soil pH at planting	Spore load		Soil pH at planting	Spore load
		$2·5 \times 10^7$	10^3		$2·5 \times 10^7$	10^5		$2·5 \times 10^7$
Untreated	4·7	97·5 (275)	12·5 (35)	7·3	42·5 (85)	0 (0)	7·5	2·5 (3)
$HgCl_2$, 0·05 %, 200 ml.	4·7	2·5 (5)	0 (0)	7·3	0 (0)	0 (0)	7·5	0 (0)
$HgCl_2$, 0·1 %, 100 ml.	4·7	15·6 (30)	0 (0)	7·3	0 (0)	0 (0)	7·3	0 (0)
$HgCl_2$, 0·1 %, 200 ml.	4·6	0 (0)	0 (0)	7·4	0 (0)	0 (0)	7·4	0 (0)
Abavit S, 1 %, 200 ml.	5·1	41·6 (60)	0 (0)	7·5	0 (0)	0 (0)	7·5	0 (0)
Abavit S, 2 %, 200 ml.	5·7	0 (0)	0 (0)	7·6	0 (0)	0 (0)	7·6	0 (0)
Abavit S, 3 %, 200 ml.	5·9	0 (0)	0 (0)	7·8	0 (0)	0 (0)	7·7	0 (0)
C.R.C., 1·25 g.	4·7	90·0 (214)	0 (0)	7·3	2·5 (3)	0 (0)	7·2	0 (0)
C.R.C., 2·5 g.	4·8	0 (0)	0 (0)	7·3	0 (0)	0 (0)	7·2	0 (0)
C.R.C., 5·0 g.	4·7	0 (0)	0 (0)	7·3	0 (0)	0 (0)	7·4	0 (0)
DA 19101, 1·25 g.	4·8	55·0 (88)	0 (0)	7·4	35·0 (58)	0 (0)	7·3	10·0 (15)
DA 19101, 2·5 g.	4·7	0 (0)	0 (0)	7·4	35·0 (73)	0 (0)	7·4	2·5 (3)
DA 19101, 5·0 g.	4·7	0 (0)	0 (0)	7·3	7·5 (10)	0 (0)	7·2	0 (0)
10101, 1·25 g.	4·7	2·7 (5)	0 (0)	7·3	0 (0)	0 (0)	7·2	5·0 (13)
10101, 2·5 g.	4·7	0 (0)	0 (0)	7·4	0 (0)	0 (0)	7·4	0 (0)

Experiment 8. In this experiment made from 12 June to 16 July 1952 the spores used for inoculating soil had been stored for 5 months. The mean air temperature during the growing period was 24·8° C. (range 13–41° C.). All the treatments giving the more satisfactory results in Exp. 7 were employed except that Aretan 3 % was substituted for the 4 % solution. Each treatment was tested in unlimed soil and in soil treated with $Ca(OH)_2$ or $CaCO_3$ at spore loads of 10^3, 10^5 and $1·5 \times 10^7$. Two pots constituted a unit for each treatment. The results obtained are stated in Table 5, except for soil treated with $CaCO_3$ in which no disease developed.

Table 5 shows that the heaviest attack occurred at the highest spore load in soil treated with $Ca(OH)_2$.

Experiment 9. Spores from fresh cauliflower roots were used to inoculate the soil used in this experiment which was made from 29 August to 7 October 1952 in a heated greenhouse with a mean air temperature of 23·3° C. (range 13–43° C.). All

TABLE 4. *Effects of soil treatments and varying spore loads using spores stored for 2 months, 1952*

Treatment (per 3000 g. oven-dried soil)	Unlimed soil			Soil treated with Ca(OH)$_2$			Soil treated with CaCO$_3$	
	Soil pH at planting	Mean % no. diseased plants and disease index Spore load		Soil pH at planting	Mean % no. diseased plants and disease index Spore load		Soil pH at planting	Mean % no. diseased plants and disease index Spore load
		$2·5 \times 10^7$	10^3		$2·5 \times 10^7$	10^5		$2·5 \times 10^7$
Untreated	4·9	94·1 (260)	92·4 (200)	7·1	97·5 (330)	97·5 (295)	7·4	92·1 (268)
HgCl$_2$, 0·05 %, 200 ml.	5·1	95·0 (303)	0 (0)	7·1	100·0 (265)	67·5 (143)	7·5	55·0 (113)
HgCl$_2$, 0·1 %, 100 ml.	5·0	86·9 (260)	12·7 (16)	7·2	100·0 (330)	95·0 (240)	7·5	63·8 (155)
HgCl$_2$, 0·1 %, 200 ml.	5·0	20·0 (28)	0 (0)	7·1	95·0 (233)	15·0 (23)	7·4	27·5 (50)
Abavit S, 1 %, 200 ml.	5·7	68·4 (230)	30·0 (53)	7·2	97·5 (202)	94·7 (249)	7·7	49·2 (88)
Abavit S, 2 %, 200 ml.	6·0	77·5 (160)	0 (0)	7·5	77·5 (155)	58·8 (136)	7·7	30·0 (73)
Abavit S, 3 %, 200 ml.	6·2	7·9 (16)	0 (0)	7·8	37·5 (60)	5·3 (8)	7·8	0 (0)
Aretan, 2 %, 200 ml.	6·1	0 (0)	0 (0)	7·6	81·8 (205)	5·0 (5)	7·8	7·9 (21)
Aretan, 4 %, 200 ml.	6·7	0 (0)	0 (0)	8·0	0 (0)	0 (0)	8·1	0 (0)
Washing soda, 10 %, 200 ml.	6·7	89·5 (176)	65·0 (138)	8·2	100·0 (278)	20·6 (39)	8·1	2·5 (5)
Orthocide, 0·4 %, 200 ml.	5·0	89·7 (232)	7·5 (8)	7·1	100·0 (223)	97·5 (331)	7·5	74·4 (143)
Orthocide, 0·8 %, 200 ml.	5·0	40·0 (58)	0 (0)	7·0	67·5 (123)	92·5 (213)	7·4	15·4 (23)
Orthocide, 1·6 %, 200 ml.	4·9	30·0 (35)	0 (0)	7·1	51·1 (89)	0 (0)	7·5	3·0 (9)
Orthocide, 3·2 %, 200 ml.	5·0	0 (0)	0 (0)	6·9	2·5 (3)	0 (0)	7·5	0 (0)
C.R.C., 1·25 g.	5·0	100·0 (320)	23·2 (36)	7·2	97·5 (293)	86·4 (165)	7·5	75·0 (168)
C.R.C., 2·5 g.	5·1	59·9 (135)	0 (0)	7·2	100·0 (320)	44·1 (55)	7·5	63·8 (120)
C.R.C., 5·0 g.	5·2	0 (0)	0 (0)	7·2	49·5 (68)	0 (0)	7·6	17·9 (21)
DA 19101, 1·25 g.	5·1	57·9 (106)	27·9 (36)	7·0	97·5 (271)	100·0 (305)	7·5	65·0 (153)
DA 19101, 2·5 g.	5·2	52·4 (93)	12·7 (13)	7·1	94·7 (227)	92·1 (222)	7·5	79·2 (181)
DA 19101, 5·0 g.	5·1	0 (0)	0 (0)	7·1	92·5 (165)	77·8 (175)	7·6	51·7 (91)

TABLE 5. *Effects of soil treatments and varying spore loads using spores stored for 5 months, 1952*

Treatment (per 3000 g. oven-dried soil)	Unlimed soil				Soil treated with Ca(OH)$_2$			
	Soil pH at planting	Mean % no. diseased plants and disease index Spore load			Soil pH at planting	Mean % no. diseased plants and disease index Spore load		
		$1·5 \times 10^7$	10^5	10^3		$1·5 \times 10^7$	10^5	10^3
Untreated	4·8	62·5 (117)	25·0 (50)	20·0 (31)	6·9	83·6 (280)	31·6 (79)	5·3 (21)
HgCl$_2$, 0·1 %, 200 ml.	4·8	0 (0)	0 (0)	0 (0)	7·0	5·0 (12)	0 (0)	0 (0)
Abavit S, 3 %, 200 ml.	6·1	2·7 (3)	0 (0)	*	7·4	2·6 (3)	0 (0)	*
Aretan, 2 %, 200 ml.	6·0	5·0 (10)	0 (0)	0 (0)	7·4	2·5 (5)	0 (0)	0 (0)
Aretan, 3 %, 200 ml.	6·3	0 (0)	0 (0)	0 (0)	7·6	2·8 (6)	0 (0)	0 (0)
C.R.C., 5 g.	4·9	0 (0)	0 (0)	0 (0)	7·0	0 (0)	0 (0)	0 (0)
DA 19101, 5 g.	4·9	0 (0)	0 (0)	0 (0)	6·9	57·2 (118)	0 (0)	0 (0)

* Test not made due to shortage of fungicide.

the treatments used in the previous experiment, with the exception of Abavit S were included in the test. In unlimed soil and in soil treated with $Ca(OH)_2$ spore loads of 10^7, 10^5 and 10^3 were employed but in soil treated with $CaCO_3$ only the highest spore load was used. Two pots constituted a unit for each treatment under each set of conditions. The results obtained are presented in Table 6.

It is clear from the results that very heavy attacks occurred except at the lowest spore loads in soil treated with $Ca(OH)_2$. As in previous experiments, fungicides were more effective at low than at high spore loads.

(*b*) *Effects of time of year on efficacy of soil treatments*

Experiment 10. To ascertain if tests could be carried out under poorer growing conditions than those prevailing when Exps. 5–9 were made, one experiment was carried out in winter (28 November 1951–30 January 1952) in a heated greenhouse with a mean air temperature of 23·3° C. (range 12–35° C.). Artificial lighting was used to supplement daylight. The spores used for inoculating soil were obtained fresh from cabbage roots. Each treatment was tested (*a*) in unlimed soil with spore loads of 10^3 and $2{\cdot}5 \times 10^7$, (*b*) in soil treated with $Ca(OH)_2$ at spore loads of 10^5 and $2{\cdot}5 \times 10^7$, and (*c*) in soil treated with $CaCO_3$ at a spore load of $2{\cdot}5 \times 10^7$. Two pots constituted a unit for each treatment under each set of conditions. The results obtained are stated in Table 7.

Table 7 shows that although very heavy attacks of the disease did not occur the heaviest attacks were in soil treated with $Ca(OH)_2$. The plants growing in limed soils developed better root systems than those in unlimed soil and this may, in part, account for the occurrence of the heavier attacks in soil treated with $Ca(OH)_2$ since the development of clubs must of necessity be slow when root growth is poor. It was again observed that treatment with fungicide DA 19101 was associated with root discoloration.

(*c*) *Comparison of results*

Comparison of the results obtained from Exps. 5 to 10 shows that attacks of different intensities occurred in different experiments even at the same spore load and that the intensity of attack influenced the effectiveness of most fungicides. However, all the experiments were not made under comparable conditions and the effects of certain factors on the intensity of attack and the efficacy of fungicides can be studied.

In Exps. 5–8 the spores obtained from cabbage roots had been stored for varying periods. Those used in Exps. 5 and 6 were collected from one source at the same time, while those used in Exps. 7 and 8 were collected from another source at a different time. In Exp. 9 spores from fresh cauliflower roots were employed. Apart from these differences the five experiments were made under similar conditions. The results obtained show that in the absence of a fungicide attacks of

TABLE 6. *Effects of soil treatments and varying spore loads using fresh spores, 1952*

Treatment (per 3000 g. oven-dried soil)	Unlimed soil				Soil treated with Ca(OH)$_2$				Soil treated with CaCO$_3$	
	Soil pH at planting	Mean % no. diseased plants and disease index. Spore load 10^7	10^5	10^3	Soil pH at planting	Mean % no. diseased plants and disease index. Spore load 10^7	10^5	10^3	Soil pH at planting	Mean % no. diseased plants and disease index. Spore load 10^7
Untreated	5·1	94·4 (307)	100·0 (375)	94·7 (322)	7·0	91·9 (297)	65·2 (210)	23·5 (38)	7·3	84·6 (228)
HgCl$_2$, 0·1 %, 200 ml.	5·2	61·6 (98)	7·8 (8)	0 (0)	7·2	64·4 (155)	25·0 (48)	0 (0)	7·3	5·0 (5)
Aretan, 2 %, 200 ml.	6·2	95·0 (208)	15·0 (20)	0 (0)	7·5	90·0 (245)	0 (0)	0 (0)	7·6	0 (0)
Aretan, 3 %, 200 ml.	6·8	64·5 (147)	0 (0)	0 (0)	7·8	10·0 (15)	0 (0)	0 (0)	7·8	0 (0)
C.R.C., 5 g.	5·2	22·5 (38)	0 (0)	5·0 (8)	7·1	32·5 (43)	0 (0)	0 (0)	7·3	0 (0)
DA 19101, 5 g.	5·2	88·5 (238)	44·3 (86)	7·7 (8)	7·3	93·2 (294)	79·4 (194)	42·9 (79)	7·4	80·6 (168)

TABLE 7. *Effects of soil treatments under winter conditions at different levels of spore load using fresh spores, 1951*

Treatment (per 3000 g. oven-dried soil)	Unlimed soil			Soil treated with Ca(OH)$_2$			Soil treated with CaCO$_3$	
	Soil pH at planting	Mean % no. diseased plants and disease index. Spore load $2·5 \times 10^7$	10^3	Soil pH at planting	Mean % no. diseased plants and disease index. Spore load $2·5 \times 10^7$	10^5	Soil pH at planting	Mean % no. diseased plants and disease index. Spore load $2·5 \times 10^7$
Untreated	4·8	28·3 (48)	0 (0)	7·0	83·0 (154)	57·8 (117)	7·4	0 (0)
HgCl$_2$, 0·05 %, 200 ml.	4·8	0 (0)	0 (0)	7·1	49·0 (84)	5·3 (6)	7·6	0 (0)
HgCl$_2$, 0·1 %, 100 ml.	4·8	0 (0)	0 (0)	7·2	48·7 (84)	0 (0)	7·6	0 (0)
HgCl$_2$, 0·1 %, 200 ml.	4·8	0 (0)	0 (0)	7·0	18·3 (26)	0 (0)	7·5	0 (0)
Abavit S, 1 %, 200 ml.	5·4	31·8 (64)	0 (0)	7·4	72·1 (135)	20·0 (23)	7·7	0 (0)
Abavit S, 2 %, 200 ml.	5·9	4·6 (5)	0 (0)	7·7	19·0 (22)	18·4 (30)	7·7	0 (0)
Abavit S, 3 %, 200 ml.	6·1	0 (0)	0 (0)	7·8	2·5 (3)	2·9 (3)	7·9	0 (0)
C.R.C., 1·25 g.	4·9	50·6 (87)	0 (0)	7·3	73·7 (153)	15·6 (22)	7·5	0 (0)
C.R.C., 2·5 g.	4·9	0 (0)	0 (0)	7·2	65·0 (95)	11·1 (11)	7·6	0 (0)
C.R.C., 5·0 g.	4·9	0 (0)	0 (0)	7·1	10·0 (10)	0 (0)	7·6	0 (0)
DA 19101, 1·25 g.	4·9	11·8 (18)	0 (0)	7·3	81·9 (179)	47·3 (89)	7·5	0 (0)
DA 19101, 2·5 g.	4·9	3·0 (6)	0 (0)	7·2	68·5 (111)	43·3 (79)	7·4	3·1 (6)
DA 19101, 5·0 g.	4·9	0 (0)	0 (0)	7·2	10·0 (13)	11·8 (24)	7·6	0 (0)

approximately equal intensity occurred when the spores were used fresh or after storage for 2 months. When spores were used after storage for 5 months, less severe attacks sometimes occurred and when the storage period was extended to 7 months the attacks were even less intense. The germination of the spores may decline during storage, but because of the difficulties encountered in observing germination it was not possible to confirm this probability. The results obtained from these five experiments also demonstrate that fungicides may be more effective when the spores have been subjected to long storage. In experiments where the spores were used fresh, or had been stored for 2 months only, treatments could be divided into more grades of efficiency than in experiments where the spores were used after prolonged storage. Treatments found to be inefficient in experiments where spores were used after long storage were also found to be unsatisfactory when the spores employed had been stored for shorter periods.

In each of the experiments made each fungicide was tested at different levels of spore load in limed and unlimed soil. The results obtained demonstrate that fungicides inhibiting infection by *P. brassicae* were more effective at low than at high spore loads. They also show that even at the same spore load fungicides were often less effective in soil treated with $Ca(OH)_2$ than in unlimed soil or when alkalinity was induced by treatment with $CaCO_3$. The plants in limed soil grew better than those in unlimed soil, but since growth was equally good when $Ca(OH)_2$ or $CaCO_3$ was employed, the lower efficiency of fungicides after an application of $Ca(OH)_2$ cannot be attributed solely to increased growth.

Study of the results obtained in Exps. 7, 9 and 10 enables a comparison to be made between tests carried out in winter and in spring or autumn. In those experiments all conditions except time of year were comparable, the spores used not having been stored for longer than 2 months. The attacks occurring under all conditions were much more severe in the spring and autumn than in the winter. Treatments which provided a high measure of control during winter were not so effective in the spring or autumn, although they proved more efficacious than treatments giving less satisfactory results in the winter test.

The data presented, while emphasizing the necessity of carrying out tests under comparable conditions when fungicides are being evaluated, indicate that if suitable precautions are taken consistent conclusions can be reached.

Consistency of results from replicates

The agreement between results from replicates was satisfactory when heavy or slight attacks occurred, but when moderate attacks developed the same close agreement was not always found.

An experiment was made to ascertain if treatment of soil in separate bulks with the same fungicides might permit of any substantial differences arising in the results obtained. In this experiment high and low spore loads were employed in unlimed soil and in soil traeted with $Ca(OH)_2$. The results obtained for the percentage number of diseased plants and for disease index showed that no substantial differences could be attributed to the preparation and treatment of separate bulks of the same soil.

RECOMMENDED SCHEDULE OF TESTING

It is recommended that fungicides to be tested should be subjected to a preliminary pot test, and those which are considered satisfactory should then be subjected to a more detailed test.

Preliminary pot test. This test should be carried out at a spore load of 10^5. Each fungicide should be tested in acid soil of pH 5·0–6·3 as well as in soil treated with $Ca(OH)_2$ to adjust the reaction to pH 7·0–7·2. Two replicates, each pot containing twenty plants, should be included.

Detailed pot test. In this test spore loads of 10^3, 10^5 and between 10^7 and $2{\cdot}5 \times 10^7$ should be employed. Each fungicide should be tested at each spore load in acid soil (pH 5·0–6·3) and in soil of pH 7·0–7·2, the reaction having been adjusted by addition of $Ca(OH)_2$. The number of replicates may vary between two and five according to the degree of accuracy desired and the space and labour available.

The following recommendations apply to the carrying out of the preliminary and detailed tests:

Soil. A light loam is satisfactory, but if desired other soil types may be included in the detailed test. The soil used should be free from contamination with *P. brassicae*.

Treatment of soil with fungicides (see p. 291). Fungicides in powder form should be applied to air-dried soil which has already been treated with $Ca(OH)_2$ if the soil reaction required adjustment. Fungicides in solution should be added after the spore suspension has been incorporated with the soil. Very thorough mixing by hand should be given to the soil after the addition of fungicides as well as after adding the spore suspension.

Spores. The spores used for inoculating soil should not have been stored for longer than 2 months. Where possible fresh spores from diseased roots should be employed.

Moisture content of soil. The soil moisture content should be maintained at 70 % of the maximum water-holding capacity by weighing the pots on alternate days and replacing the water lost.

Temperature. Tests should be made at a mean air temperature of 23° C., but those in an acid soil may also be made at 18° C.

Time of year. Tests should be made only under conditions which permit of good plant growth. It is difficult to secure heavy attacks of the disease during winter even when daylight is supplemented by artificial lighting.

Duration of test. A period of 4–5 weeks is required but the plants should continue to be grown until there are well-developed clubs on the roots of those in soil not treated with any fungicide.

Assessment of the efficiency of fungicides. The values obtained for the percentage number of diseased plants and for disease index should be used to compare the efficacy of each fungicide with that of a standard treatment. It is suggested that treatment with 200 ml. of a 0·1 % solution of mercuric chloride per 3000 g. of oven-dried soil should be accepted as the standard.

DISCUSSION

It has been shown in studies of the epidemiology of club-root disease that in acid soils the level of spore load (within the limits of 10^3 to $2{\cdot}5 \times 10^7$ spores per g. of soil) does not influence the number of diseased plants when favourable conditions for an attack and for good growth of the host are provided, although under less favourable conditions spore load does exercise an effect (Colhoun, 1953*a*). It was also found that at moisture contents and temperatures favourable for infection a heavy attack develops in alkaline soils only in the presence of a high spore load. While these conclusions have been confirmed in the present investigation it has also been shown that they are applicable only when the spores used for inoculating soil have not been stored for lengthy periods. If spores are not used in a reasonably fresh condition the attack in both acid and alkaline soils varies with spore load, is heaviest at the highest spore load and becomes less severe as the storage period is lengthened. The results now obtained may indicate that as the period of storage of spores lengthens either the percentage number of the spores capable of germinating declines or the amoebae from such spores are less efficient in bringing about infection than are those from fresh spores.

The importance of the viability of the spores used for inoculation was appreciated by Bremer *et al.* (1937) who tested viability by plasmolysis. They appreciated that this method did not provide an accurate measurement of the germinating capacity of the spores but it was adopted because spore germination is very irregular and difficult to observe. Bremer and his co-workers reported that, under the conditions of storage they employed, spores retained their power of infection unchanged for more than a year. Macfarlane (1952) found that spores became non-viable after storage for 6 months. Although the writer has been unable to obtain data regarding the germination of the spores used in different experiments it is clear from the results presented that some spores remained viable for at least 7 months after being placed in store.

It is evident from the results obtained in this study that the efficacy of fungicides in controlling club-root disease is dependent upon a number of factors which include spore load, duration of spore storage, time of year and temperature. If consistent results are to be obtained in tests made on different occasions these factors must be comparable.

Bremer *et al.* (1937) demonstrated that the efficacy of fungicides is influenced by the spore load present, and that in two series of tests a fungicide may show higher efficiency at a lower spore load, although the severity of the attack in the control pots may be equal at the different spore loads employed. The results now obtained show substantial agreement with these conclusions since it was found that fungicides usually show greater efficiency in soils with a low spore load, irrespective of whether or not the degree of attack in untreated soil is affected by variations in spore load. It is also now clearly proved that the period during which spores are stored is of great importance when the efficiency of fungicides is being tested. The efficacy of fungicides increases with extension of the storage period of spores and is least when they are used fresh from diseased roots or after only a short period of storage.

The effect of time of year on the effectiveness of fungicides was considered by Bremer *et al.* (1937). They found that the less efficient materials were more effective in winter than in summer tests where heavier attacks and better growth of the host occurred. This finding is now confirmed.

The observation that fungicides are often less effective in soil treated with $Ca(OH)_2$ than in unlimed soil is of interest. Bremer (1924) placed sections of infected roots in tubes containing a phosphate buffer solution varying from pH 5·4 to 8·0 to which was added a standard amount of mercuric chloride solution. Above pH 7·0 he found that there was a marked increase in the percentage of live spores as determined by plasmolysis, and at pH 8·0 the toxic effect of the fungicide was almost completely inhibited. Bremer concluded that spores are more resistant to harmful external influences in soil with a faintly alkaline reaction. By comparison with Bremer's finding the results now obtained suggest that spores may be less affected by most fungicides in alkaline soils where alkalinity is induced by the soluble base, $Ca(OH)_2$, than where the more insoluble salt, $CaCO_3$, is employed.

As a result of field trials made in many countries it is now generally accepted that commercial control of club root is obtained by use of mercuric chloride. Many workers have stated that calomel (mercurous chloride) also provides good control. It is of interest to note that mercuric chloride and the proprietary fungicide C.R.C., which contains calomel, are among the most effective materials tested in this investigation.

The results presented show that fungicides can be evaluated by comparing their efficacy with that of a standard treatment in controlled experiments. It is believed that such comparisons can be made more fairly in tests under controlled conditions than in the field. Such a pot test has the advantage that results can be obtained inside a period of 4–5 weeks and, if desired, different soil types may be employed. Definite information can also be obtained regarding the efficiency of fungicides in the presence of different spore loads and this may assist in explaining any anomalous results arising later in field trials. It is not suggested that the introduction of such a test eliminates the necessity of carrying out field experiments. The pot test should enable large numbers of fungicides to be readily assessed in the minimum of time and those which are considered satisfactory should then be tested in the field. The field test enables rates and methods of application to be studied further as well as permitting observations on phytotoxicity to be made. Some information on phytotoxicity can be provided by pot experiments, but the conclusions reached may not be applicable in the field especially if the methods of application differ. Results obtained from field experiments made in Northern Ireland (unpublished work) show, for example, that toxicity is exhibited when various species of Brassicae are treated in dibble holes at transplanting with Aretan 2% at the rate of a half pint per plant. The same concentration of this fungicide thoroughly incorporated with the soil in pot experiments was not phytotoxic.

It gives the writer much pleasure to record his thanks to Prof. A. E. Muskett for his continued interest and helpful criticism. Mr G. I. Janeway rendered valuable assistance in the large amount of routine work.

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STUDIES IN THE ANTAGONISM BETWEEN *BLASTOMYCES LUTEUS* AND SPECIES OF *VERTICILLIUM*

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(With 1 Text-figure)

Blastomyces luteus has been shown to be antagonistic in culture to both *Verticillium albo-atrum* and *V. dahliae* over a wide range of temperature and irrespective of the pH value of the medium. When *B. luteus* is grown on Dox's and potato-dextrose solutions it produces an exudate which, added to agar media, has an inhibiting effect upon the growth of these two species of *Verticillium*. The effectiveness of this inhibiting material is slightly reduced by boiling for 15 min.

When 'spent' Dox's liquid medium, which has supported the growth of *B. luteus*, is injected into tomato and antirrhinum seedlings inoculated with *Verticillium* no control of the disease is effected, but when *B. luteus* mycelium is added to soil infected with *Verticillium*, marked reduction in the incidence of disease results when the antagonistic organism and the pathogens have co-existed in the soil a sufficient length of time for the exudate of the former to be effective upon the development of the latter.

During cultural work involving *Verticillium albo-atrum* and *V. dahliae*, a fungal contaminant which proved to be antagonistic to these two species appeared on one plate of agar. This fungus was identified by Mr E. W. Mason, of the Commonwealth Mycological Institute, as *Blastomyces luteus* Cost. & Roll. The work here reported was designed to determine the interaction between the two named species of *Verticillium* and *B. luteus*, both in culture and in phytopathological experiments, in order to investigate the possibility of the use of *B. luteus* in the control of *Verticillium* wilt diseases.

Although the potential importance of antibiotic substances in inhibiting the development of plant diseases has been recognized for some time by plant pathologists, these substances, as yet, play only a very insignificant role in control measures. The relevant literature on this subject was reviewed by Wood (1951).

Cultural studies

In all the cultural experiments Petri dishes (9 by 2 cm.) filled with agar media to a depth of 3 mm. were used, and these plates were inoculated simultaneously with mycelium of either *V. albo-atrum* or *V. dahliae* and of *B. luteus*, the points of inoculation being approximately 4 cm. apart. Six plates of each of the following media: Dox's, potato-dextrose, potato-extract, oatmeal-extract, 2·5 % malt-extract, Czapek's, Brown's, 2 % prune-extract and sugar beet-extract agars, were inoculated with *V. albo-atrum* and *B. luteus* and another six of each with *V. dahliae* and *B. luteus*. Suitable control plates of each medium inoculated with only one of each of the respective fungi were kept, and all were incubated at 22·5° C.

On all the control plates the species of *Verticillium* grew fairly rapidly, the diameter of the colonies on Dox's medium reaching approximately 60 mm. after 15 days. *B. luteus*, however, developed so slowly that at the end of 15 days the colonies were never more than 15 mm. in diameter. Where both organisms had been inoculated on to a single plate the species of *Verticillium* present invariably grew normally for 2–3 days, after which, on all media, it became affected by the presence of the slowly developing *B. luteus*. An inhibition zone, varying in width from 3 to 8 mm. was formed between the two, although the *Verticillium* sp., growing faster, frequently surrounded the colony of *B. luteus*, though separated from it by a clear zone. The pH of the medium in this clear region was always the same as that of the remainder of the plate, showing that, as Newhook (1951) found when investigating the antagonism between *Actinomyces* and *Botrytis cinerea*, pH change was not causally connected with the inhibition.

Effect of temperature

In order to test the effect of temperature upon the degree of antagonism between these organisms two separate series, each consisting of four plates of both Dox's and potato-dextrose agars, were inoculated, one series with *V. albo-atrum* and *B. luteus*, the other with *V. dahliae* and *B. luteus*, suitable control plates being simultaneously inoculated each with one of these three fungi. These pairs of series were duplicated 5 times, each pair being incubated at one of the following temperatures, 10·0, 15·0, 20·0, 22·5 and 25·0° C. Since *V. dahliae* grows at 30·0° C., while *V. albo-atrum* does not (Isaac, 1949) one additional series was inoculated with the former and *B. luteus* for testing at this temperature.

On every plate inoculated with both *B. luteus* and a *Verticillium* sp. an inhibition zone was formed at every temperature tested, while normal growth of the controls invariably occurred.

Effect of pH *of the medium*

Since the media containing potato-dextrose and buffered Dox's (normal Dox's plus sodium and potassium phosphates) maintain a constant pH value during the growth of both *V. albo-atrum* and *V. dahliae* (Isaac, 1949) these two, as well as normal unstable Dox's were used to determine the effect of the pH of the media upon the interaction of these organisms. The three media were adjusted to the following range of pH values: 3·0, 3·6, 4·6, 5·3, 6·4, 7·2, 8·0, 8·6 and 9·6 respectively, using sodium hydroxide and hydrochloric acid, both of normal strength, for ordinary Dox's, and of twice normal strength for the two buffered media. Series of four plates of each medium at each pH value were prepared for *V. albo-atrum* and *B. luteus* and a similar series for *V. dahliae* and *B. luteus*, and these, with suitable controls consisting of three series of plates at each pH value each inoculated with one of the three fungi, were incubated at 22·5° C. and kept under observation for 3 weeks.

On every plate inoculated with two organisms, an inhibition zone formed between them while the control colonies developed normally, thus proving that antagonism between these organisms occurs irrespective of the pH value of the media on which they grow.

Effect of 'spent' media

To determine whether *B. luteus* when grown on liquid media leaves any *Verticillium*-antagonizing residue Dox's and potato-dextrose solutions were inoculated with spore suspensions of *B. luteus* and incubated for 15 days at 22·5° C. The mycelial mat formed during this period was then removed and the 'spent' media passed through a sterile filter, half the filtrate being then boiled for 15 min.

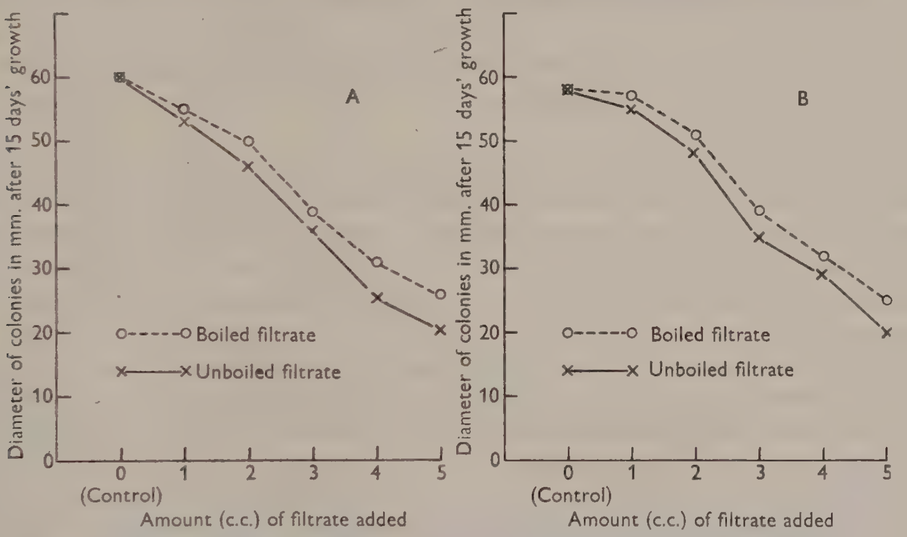

Fig. 1. Effect of addition of a 'spent' Dox's medium filtrate upon growth of *V. albo-atrum* (A) and *V. dahliae* (B).

Six series, each consisting of eight plates containing 15 c.c. of Dox's agar, were prepared. One series served as a control while to each of the other five series, before the agar solidified, were added 1, 2, 3, 4, and 5 c.c. respectively of unboiled 'spent' Dox's medium. A precisely similar set of forty-eight plates was prepared for the testing of the boiled filtrate, and the whole experiment was duplicated using potato-dextrose agar together with the unboiled and boiled 'spent' potato-dextrose solutions. Half the plates in each series were inoculated with *V. albo-atrum* and half with *V. dahliae*, and all were incubated at 22·5° C. for 15 days, after which the mean diameter of each series was recorded. In Fig. 1 above, which summarizes the results, only data for growth on Dox's agar medium are shown since the reactions on both media were similar.

Fig. 1 shows that when solutions containing the staling product of *B. luteus* were added to normal media inoculated with the two species of *Verticillium* the development of both of these species was greatly reduced, suggesting that an inhibitory substance had been left by *B. luteus* in both Dox's and potato-dextrose solutions. The effectiveness of this substance was only slightly reduced by boiling for 15 minutes.

PATHOGENICITY STUDIES

In view of the above results the possibility of using *B. luteus* to control diseases caused by *V. albo-atrum* and *V. dahliae* was investigated in the following manner:

A. Each plant of a series of twenty-five tomato and twenty-five antirrhinum seedlings was inoculated just above ground-level, by means of a hyperdermic syringe, with conidia of *V. albo-atrum*, and an exactly similar series with conidia of *V. dahliae*. Immediately following upon this, and on each subsequent day, 2 c.c. of the filtrate of 'spent' Dox's medium which had supported the growth of *B. luteus*, obtained as described above, were injected with the syringe into each of twenty of both inoculated plants in each series, the remaining five in each set being kept as controls.

The results proved that the 'spent' medium had no therapeutic effect, since during the next 3–12 weeks forty-five of the tomato and forty-eight of the antirrhinum plants developed disease symptoms, indicating that although a substance inhibiting the growth of the two species of *Verticillium* in culture is present in the filtrate this had no such effect on the development of the pathogens in host plant tissues.

B. A John Innes compost, contained in two series, each consisting of twenty-seven pots (10 in. diameter) was mixed with a 7-week-old wheat-grain culture of *V. albo-atrum* and *V. dahliae* respectively every month from December to June inclusive, and to eighteen pots of each series a similar culture of *B. luteus* was immediately added. Nine pots of each series, inoculated with a *Verticillium* sp. only, thus served as controls. Each month also a series of three similar pots of soil was inoculated with *B. luteus* only, a further series of three being uninoculated so that both series served as additional controls.

Immediately after the soil was inoculated in June attempts were made to re-isolate the respective fungi from every pot in all the series, using the water dilution method employed by Brierley, Jewson & Brierley (1927). For plating, 1 c.c. of 1:10,000 or 1:20,000 dilution was added to 15 c.c. of medium and ten plates were poured for each series. The results obtained for the soil infected with *Verticillium* only were identical with those recorded earlier (Isaac, 1953), i.e. many colonies were obtained from soil inoculated within the preceding 4 months, while soil inoculated earlier than this yielded no isolates. Where *B. luteus* was added alone or with either *V. albo-atrum* or *V. dahliae* the former was recovered from every series, but both species of *Verticillium* were re-isolated only from soil inoculated in April, May and June, and where either of these organisms appeared on plates, together with *B. luteus*, their growth was markedly inhibited by the latter.

After these attempts at isolation of the fungi from the soil in June, the whole range of pots, irrespective of the date of their inoculation, was planted with tomato and antirrhinum seedlings. Two-thirds of the pots in each series that had been inoculated in any one month were planted with one tomato seedling per pot, and the remaining one-third with two antirrhinum seedlings per pot. The total numbers of these test plants were therefore equal, and their distribution through the seven series of pots which had been previously inoculated in seven successive months was identical. Thus twelve of each type of seedling were planted in soil which had been infected each month with each of the species of *Verticillium* plus *B. luteus*, six of each type in soil inoculated each month with *V. albo-atrum* and *V. dahliae* respectively, two of each in soil inoculated each month with *B. luteus* only, and two of each type in uninoculated soil. The results are summarized in Table 1.

TABLE 1. *Effect of* B. luteus (B.l.) *upon* V. albo-atrum (V.a.a.) *or* V. dahliae (V.d.) *diseases of tomatoes* (*T*) *and antirrhinums* (*A*) *planted in June*

		Number of plants wilted in the series inoculated in													
	Total no. of each plant in each series	Dec.		Jan.		Feb.		Mar.		Apr.		May		June	
Fungus		T	A	T	A	T	A	T	A	T	A	T	A	T	A
V.a.a.+*B.l.*	12	0	0	0	0	0	1	0	2	8	10	9	12	8	11
V.a.a. only	6	4	5	4	6	4	6	5	4	6	6	6	5	6	6
V.d.+*B.l.*	12	0	0	0	0	0	0	2	1	6	9	4	8	8	12
V.d. only	6	3	5	3	6	5	5	4	6	5	6	6	6	6	6
B.l. only	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
No fungus	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The appropriate species of *Verticillium* was isolated from every wilted plant, and since wilting did not occur in plants grown in soil inoculated with both pathogen and antagonist unless such inoculation had occurred at least 3 months prior to planting, the results suggest that *B. luteus* has a controlling effect upon diseases of tomato and antirrhinum caused by species of *Verticillium* only when the two have co-existed in the soil for a period of sufficient duration for the antagonistic effect of *B. luteus* upon the pathogen to become manifest.

Isaac (1953) indicated that after approximately 5–6 months in soil the hyaline vegetative mycelium and conidia of *V. albo-atrum* and *V. dahliae* die out, and that these fungi then persist as carbonized resting-mycelium and microsclerotia respectively, which are not recoverable from the soil when the water-dilution isolation technique is employed. However, when a susceptible host plant is placed in such soil, infection from these resting bodies usually occurs, presumably consequent upon their germination, which may possibly be stimulated by the presence of the host plants. The present work confirms this finding for in soil infected by either

V. albo-atrum or *V. dahliae* alone the tomato and antirrhinum seedlings showed heavy incidence of disease irrespective of the date at which the soil in which they were grown was inoculated. On the other hand, in the presence of *B. luteus* heavy infection occurred when planting followed fairly closely upon inoculation, while when planting was delayed for a period of 3 months or more after inoculation, the incidence of disease symptoms decreased proportionally. Since immediately subsequent to inoculation there was an abundance of vegetative mycelium and conidia in the soil a high rate of infection occurred. With the passage of time these agents of infection progressively diminished leaving an increasing proportion of normally viable resting bodies, a phenomenon which coincided with a diminution of disease symptoms. It therefore seems likely that *B. luteus* living saprophytically in the soil either produced some antibiotic material which inhibits the normal germination of the resting-mycelium and microsclerotia of *V. albo-atrum* and *V. dahliae* respectively, or stimulates the production of some other very resistant resting bodies which remain dormant in its presence. Though support is lent to this latter suggestion by the report of Vankat Ram (1952) that *Fusarium solani* formed resistant chlamydospores in the presence of soil bacteria, as a result, he suggested, of their production of antibiotics, the occurrence of such hitherto unknown highly resistant resting bodies must be regarded as highly problematical. However, this suggestion will be further explored by the writer, and will be the subject of a later report.

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THE RELATIONSHIP BETWEEN NICKEL TOXICITY AND IRON SUPPLY

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The influence of varying levels of iron and substrate pH on the uptake of nickel and the intensity of toxicity symptoms in oat plants have been investigated using sand- and water-culture techniques.

Nickel-toxicity symptoms (both necrosis and chlorosis) are less severe when the concentration of iron in the nutrient solution is high. The reduction in degree of necrosis is related to a reduced content of nickel in the leaf blades, whilst that of chlorosis is related to the Ni/Fe ratio in the leaf blades—an internal antagonism being indicated in the latter case.

A reciprocal relationship exists between the nickel and iron contents of the leaf blades; the nickel content is materially reduced by high concentrations of iron in the nutrient solution, and the iron content by nickel, the former being the more pronounced effect.

Uptake of nickel increases with increasing pH for a constant iron level in the substrate, although the degree of necrotic symptoms is similar over pH range 4–7. Iron uptake is reduced by both nickel and increasing pH and results in chlorosis at pH values of 5·5 and above.

For a constant level of iron supply the phosphate content of the stem extracts is higher the greater the degree of nickel toxicity; the phosphorus status of the plant may be a factor in producing nickel toxicity but if so, it has to be considered in relation to other factors.

Introduction

It is generally recognized that relatively low concentrations of nickel are toxic to plants. Excessive nickel occurs naturally in certain Aberdeenshire soils which have been examined by the authors (Hunter & Vergnano, 1952), who also investigated the effect of nickel on oat plants, including changes produced in leaf anatomy (Vergnano & Hunter, 1952). Briefly, the symptoms of nickel toxicity are chlorosis—which responds to leaf applications of ferrous sulphate—and necrosis in the form of white longitudinal leaf stripes.

Many authors have drawn attention to the production of iron deficiency chlorosis by excessive concentrations of metals, and the subject has been reviewed by Hewitt (1951). Copper, cobalt, nickel, chromium, manganese and zinc are all known to induce iron deficiency when present in excess; Hewitt (1948*b*, *c*) has investigated the action of all these when supplied in nutrient solution and Millikan (1947, 1948) has dealt with all except chromium.

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The literature provides considerable evidence that the iron deficiency induced by metals is generally caused not by an insufficiency of iron in the plant but by the unavailability of the iron in the tissues. The total-iron content of leaves is not therefore necessarily related to the iron status of the tissues, the total-iron content in chlorotic leaves being frequently equal to or more than that of normal (Nicholas, 1950). Thorne & Wallace (1944), however, consider that the total-iron content may be significant if measured on the basis of leaf area rather than of dry matter. Soluble fractions of the iron content appear to be of more value in determining iron availability; the water-soluble iron content, for example, has been successfully used by Nicholas (1950), the iron content of the expressed sap by Rogers & Shive (1932), and the iron soluble in 0·5 N hydrochloric acid by Lindner & Harley (1944).

The importance of the ratio of iron to toxic metal (mainly Fe/Mn) in plant tissues has been emphasized by a number of writers (for example: Twyman, 1946), and Somers & Shive (1942) have suggested that iron deficiency is equivalent to manganese toxicity and vice versa. Other authors admit the importance of the ratio, but contend that the total concentration present must also be considered. Though the general application of the Fe/Mn ratio in interpreting results has been justifiably criticized, for example by Bennett (1945), and Hewitt (1951), there is little doubt that the ratio concept is a useful approach to the problem of internal iron availability.

As mentioned above, however, a reduction in total iron content may also be important; this has been observed by Kriel (unpublished: quoted by Bennett, 1945) in tomato plants receiving excessive manganese, and Sideris (1950), using radioactive iron, has found that translocation of iron from root to leaves is considerably reduced by manganese. It appears, then, that whilst an internal reduction in iron utilization is primarily responsible for the chlorosis, a further effect resulting in reduced absorption or translocation of iron may be operative in some instances.

Though it has been suggested that trace-element toxicities are simply induced iron-deficiency, it is generally accepted that each trace element produces other toxic effects which are specific and unrelated to iron supply (Hewitt, 1946, 1947, 1948*a–c*; Millikan, 1947, 1948; Nicholas, 1950; Hunter & Vergnano, 1952, 1953; Vergnano & Hunter, 1952); the white necrotic leaf stripes being the specific toxic effect of nickel in oat plants.

In preliminary experiments carried out by the authors, however, both the chlorotic and necrotic symptoms produced in oat plants by excessive nickel were found to be less intense when the nutrient solution contained high concentrations of iron. It was observed also that the changes in leaf structure of oat plants caused by severe iron deficiency were very similar to, though less severe than, those produced by nickel toxicity (Vergnano & Hunter, 1952). It seemed possible, therefore, that the necrosis produced by nickel was a severe localized form of iron deficiency. In view of these observations and the results published by other authors, the nickel-iron relationship was further investigated in the experiments described below.

EXPERIMENTAL

Sand-culture experiments were carried out in a bird-proof cage during the summer. Oat seeds (variety Victory) were sown in fine quartz sand (32 % held by a 30-mesh and 97 % by a 60-mesh British Standard Sieve), purified by successive washings with a hot solution containing 15 % hydrochloric acid and 0·5 % oxalic acid. The containers were $4\frac{1}{2}$ in. glass pots (four per treatment), the drainage holes of which were covered by a thin layer of acid-washed glass wool. Before use, hydrogen ions adsorbed on the sand were removed by flushing the pots intermittently for 4 days with the nutrient solutions (without nickel) to be subsequently supplied.

Nutrient solutions were supplied in adequate amounts twice daily from sowing, the sand being flushed with water before the nutrient solutions were given. More water was supplied as required.

The nutrient solutions were made by diluting measured volumes of stock solutions to a predetermined volume. The composition (per litre) of the stock solutions (and volumes of these required per 10 l. of nutrient solution) were: $NaNO_3$, 250 g. (15 ml.); $Na_2HPO_4.12H_2O$, 50 g. (20 ml.); K_2SO_4, 90 g. (20 ml.); $Ca(NO_3)_2.4H_2O$, 430 g. (15 ml.); $MgSO_4.7H_2O$, 250 g. (15 ml.). The basic composition in p.p.m. of the nutrient solutions was therefore: nitrogen, 138; phosphorus, 8·7; potassium, 81; calcium, 110; magnesium, 37. Manganese, copper and boron were given from a stock solution containing 0·98 g. $MnSO_4.4H_2O$, 0·04 g. $CuSO_4.5H_2O$ and 0·35 g. H_3BO_3 per litre, 10 ml. being included in every 10 l. of nutrient solution; this supplied manganese at the rate of 0·24 p.p.m. in the nutrient solutions, copper at 0·01 p.p.m. and boron at 0·06 p.p.m.

Iron was supplied to the appropriate nutrient solutions from a stock solution of ferric citrate (6·40 g./l.). Jacobson (1951) has recommended ferric potassium ethylenediamine tetra-acetate as a useful source of available iron in nutrient solutions, and Hunter (1950–1) has confirmed that it is superior to ferric citrate for tomatoes in water culture. When the significant treatments in the experiments described below were repeated using the ferric complex instead of ferric citrate, identical results were obtained. When iron was not supplied in the experiments, some iron was available to the plants from traces remaining in the purified sand, from the distilled water (which was not glass distilled) and from the chemicals used to make the nutrient solutions, but the amounts obtained were not sufficient to prevent development of severe iron-deficiency symptoms in the plants. Nickel was supplied as nickel sulphate.

Sufficient sulphuric acid was included in all the nutrient solutions to give a pH of 5·5, but when the solutions were in contact with the plant roots, the pH gradually increased to about 6·5.

Two distinct experiments were carried out in each of which three levels of iron (0, 1·2, 12 p.p.m.) and three of nickel (0, 2·5 and 10 p.p.m.) were supplied in solutions of the basic composition given above. The essential differences between the nine treatments are shown in Table 1.

Nutrient solutions of the same basic composition and containing the appropriate concentration of iron (and no nickel) were supplied to the plants in Exp. A until the $\frac{1}{4}$ in. shoot stage, and in Exp. B until iron-deficiency symptoms first developed in the no-iron treatments (12 days after $\frac{1}{4}$ in. shoot stage); thereafter, the plants received solution of the same composition as these, but with nickel included where appropriate. In this way, nickel was supplied to young plants in Exp. A and to older plants (of established iron-status) in Exp. B.

The plants were grown for 36 days after germination. Symptoms were noted, average heights determined, and the plants harvested by cutting off $\frac{1}{4}$ in. above the sand; roots were discarded. Fully expanded (but not senescent) leaf blades of the plants were combined in one sample from all pots in each treatment. These were dried and iron and nickel contents determined; the middle thirds of stems (including leaf sheaths) of plants from each pot in each treatment were extracted in a Waring blendor with Morgan's reagent and the concentrations of nitrate, phosphate, potassium, calcium and magnesium determined in the extracts—the results being used as an index of the nutrient status of the plants. The analytical results and other data are reported in Table 1; similar nutrient status results were obtained from the four pots of each treatment and the averages of the values found are given.

In the second part of the investigation in which the effects of pH on the uptake of nickel at a constant level of iron supply were studied, water culture was employed in preference to sand culture, since variation in pH of the nutrient solution could be more readily measured and corrected.

The composition of the nutrient solution was the same as that used in sand culture and ferric citrate was used as the source of iron. The containers used were 5 l. glazed crocks. The solutions, which were changed every 4 days, were aerated continuously. The aerator assembly consisted of two glass tubes—an inner tube terminating in 3 in. of narrow bore capillary tubing and a wider outer tube sealed at the bottom but provided with two pairs of holes 1 and 9 in. from the lower end. Air passed into the inner tube displaced solution in the outer tube, forcing it out through the upper set of holes. Fresh solution was drawn in at the lower set and in this way, in addition to aeration, efficient circulation of the solution was achieved.

The plants were supported on a waxed cotton gauze fixed to the lower side of a bitumen-painted wooden ring ($\frac{1}{4}$ in. thick) of slightly smaller diameter than the crock (7 in.). Difficulties arose when normal methods of germination and transfer were employed in the production of the large number of plants of equal vigour required for the experiment, and consequently the following technique was adopted. Eighty seeds were 'sown' in each ring and the ring buried to a depth of about an inch in a 9 in. clay pot containing sand, which was watered normally. After 4–5 days the seeds had germinated and variation in size or vigour of plants was remarkably slight. When the plants were about $\frac{1}{2}$ in. high the ring, complete with plants, could be readily removed without damage to the roots by immersing the clay pot in water. After washing the roots in distilled water, the plants were immediately transferred to crocks containing the appropriate nutrient solution. The sand which was retained on the ring gave additional support to the plants and successfully prevented algal growth in the solutions by cutting down the amount of light entering the crocks.

The basic nutrient solution used supplied iron at 1·2 p.p.m. and at each pH range there were two treatments. For one of these the solution contained 1·2 p.p.m. nickel; for the other no nickel was added. Two crocks per treatment were used. Addition of 0·25 % H_2SO_4 was made twice daily to keep the pH of the cultures within the desired limits which were: 4·0–4·5, 4·5–5·0, 5·0–5·5, 5·5–6·0, 6·0–6·5, 6·5–7·0, 7·0–7·5.

Symptoms were noted at intervals, average heights determined, and plants harvested at the end of 15 days. The analytical results and other data are set out in Table 4; once again similar results were obtained for the two pots of each treatment, the averages of the values found being given.

When a parallel experiment was run using the ferric complex, comparable symptoms and levels of nickel in the toxic material were recorded. At pH levels above 6 the complex appeared to be a better source of iron than the citrate as judged by the uptake of iron by the no-nickel plants.

RESULTS

In Exp. A, all the plants receiving the iron-deficient solution were chlorotic and poorly developed; with no nickel, the chlorosis ranged from a light green interveinal form to a pale yellow diffuse type; with 2·5 p.p.m. nickel, the chlorosis was as in the no-nickel plants, but white necrotic areas in stripes and bands were also prominent in the leaves; with 10 p.p.m. nickel, both chlorosis and necrosis were extremely severe, and the plants were stunted with many leaves unfolded. With 1·2 p.p.m. iron in the nutrient solution, the plants were considerably less affected than those receiving no iron; with no nickel, they were normal; with 2·5 p.p.m. nickel, there was no chlorosis but the white striping and banding was present though less widespread than when no iron was supplied; with 10 p.p.m. nickel both chlorosis and necrotic striping and banding were as severe as with the no-iron, 10 p.p.m. nickel treatment. With 12 p.p.m. iron and no nickel in the nutrient solution, the plants were normal though slightly larger than with 1·2 p.p.m. iron; with 2·5 p.p.m. nickel, they were similar to, but larger than, the plants receiving the same level of nickel and 1·2 p.p.m. iron; with 10 p.p.m. nickel, chlorosis was less severe than in plants receiving the same level of nickel and 1·2 p.p.m. iron, but the degree of necrosis was the same.

In Exp. B the plants receiving no iron were similar in appearance to those in Exp. A, except that chlorosis was less severe in the 2·5 p.p.m. nickel treatment of Exp. A. The plants receiving 1·2 p.p.m. iron in the nutrient solution were also similar to those of the same group in Exp. A, except that the plants receiving 2·5 p.p.m. nickel were slightly chlorotic in addition to being necrotic. With 12 p.p.m. iron supplied, the plants without nickel were normal; with 2·5 p.p.m. nickel they were non-chlorotic and slightly necrotic, and with 10 p.p.m. nickel, chlorotic (as in Exp. A) and necrotic (less severely than in Exp. A). These results are summarized in Table 1.

TABLE 1. *Effect of level of iron supply on nickel toxicity*

Exp.	Treatment no.	Concentration (p.p.m.) in nutrient solution		Average height (cm.)	P.p.m. in stem extract					P.p.m. in leaf dry matter		Degree of symptoms*	
		Ni	Fe		NO_3	PO_4	K	Ca	Mg	Ni	Fe	Chlorotic	Necrotic
A	1	0	0	22	291	36	292	21	16	6·3	64	H	O
	2	2·5	0	20	248	81	300	33	17	226	62	H	H
	3	10	0	11	167	105	319	31	16	637	63	VH	VH
	4	0	1·2	24	266	22	273	20	12	5·4	120	O	O
	5	2·5	1·2	19	223	63	315	25	17	147	88	O	M
	6	10	1·2	11	148	105	308	32	13	723	79	VH	VH
	7	0	12	28	266	12	300	20	11	3·9	147	O	O
	8	2·5	12	23	254	27	335	25	14	127	144	O	M
	9	10	12	15	167	90	319	34	15	524	160	H	VH
B	1	0	0	28	223	33	195	21	16	6·5	50	H	O
	2	2·5	0	25	192	45	191	25	17	275	42	VH	H
	3	10	0	17	186	57	226	24	15	548	41	VH	VH
	4	0	1·2	30	223	26	218	19	12	4·0	95	O	O
	5	2·5	1·2	27	241	36	245	22	14	105	59	L	M
	6	10	1·2	17	167	63	261	23	14	480	59	VH	VH
	7	0	12	32	210	11	222	17	11	4·2	101	O	O
	8	2·5	12	30	192	12	245	22	12	77	75	O	L
	9	10	12	20	142	26	237	24	14	365	90	H	H+

* On scale: nil, low, moderate, high, very high; further differentiation is indicated by plus signs.

The average height data show, as expected, that the effect of nickel was more pronounced when nickel was supplied to plants from an early stage. They also show that addition of iron produced better plants, though nickel reduced plant growth at each iron level. Nickel decreased the nitrate content of the stem extracts irrespective of iron level, and increased the phosphate content, which was reduced by addition of iron. The calcium content was increased by nickel but was almost unaffected by iron. The potassium and magnesium contents were little affected by additions of nickel and iron. The only change of any magnitude was in the phosphate levels, an increase in degree of nickel toxicity symptoms being associated at a constant iron level with an increase in the phosphorus content of the extracts and presumably the tissues in general.

Both nickel and iron additions were reflected in the leaf-blade content of these elements. The iron content in the leaf blades of the no-iron treatment plants was surprisingly large. The magnitude of the nickel figures is well illustrated by comparison with the iron contents. In both experiments the nickel content was almost invariably reduced by iron additions. In Exp. A, material reduction in iron content seldom resulted from additions of nickel, but in Exp. B the iron content was higher in the leaf blades of the no-nickel plants than in those of the plants receiving nickel; the reduction in iron content by nickel was less pronounced than the reduction of nickel content by iron. The nickel contents of the leaf blades in Exp. A were higher than those from corresponding treatments in Exp. B—no doubt the result of application from an earlier stage. The iron contents in Exp. A were also higher than those in Exp. B, a feature possibly related to the small effect of nickel on iron content and to the larger size of the plants in Exp. B. The changes in nickel concentration in the leaf blades can often be associated with changes in size of the plants but may also indicate an absorption relationship between nickel and iron.

TABLE 2. *Relation between nickel and iron contents and degree of necrosis*

Degree of necrotic symptoms	Treatment no.	Concentration (p.p.m.) in leaf dry matter Ni	Fe	Ni/Fe
Very high	3B	548	41	13·4
	3A	637	63	10·1
	6A	723	79	9·2
	6B	480	59	8·1
	9A	524	160	3·3
High+	9B	365	90	4·1
High	2B	275	42	6·5
	2A	226	62	3·6
Moderate	5B	105	59	1·8
	5A	147	88	1·7
	8A	127	144	0·88
Low	8B	77	75	1·0
Nil	1A	6·3	64	0·98
	1B	6·5	50	0·13
	4A	5·4	120	0·045
	4B	4·0	95	0·042
	7B	4·2	101	0·042
	7A	3·9	147	0·027

The degree of nickel toxicity symptoms (both chlorotic and necrotic) was associated with iron supply, being generally reduced where the iron level in the solution was increased. This was particularly noticeable at the 2·5 p.p.m. nickel level; the effect of 10 p.p.m. nickel was apparently so great that an increased supply of iron was relatively ineffective in reducing it.

Although the treatments were not replicated the trends occurring in both experiments clearly indicated the relationships involved.

When the nickel contents of the leaf blades are tabulated with the degree of necrotic symptoms (Table 2), it is seen that they are related without exception. The reduction in degree of necrosis with iron additions is therefore indirect, rising from a reduction in nickel content produced by iron. There is also some relationship between the degree of necrosis and Ni/Fe ratio, but this also is probably indirect.

The nickel-iron-chlorosis relationship was similarly examined (Table 3). Here neither nickel nor iron content is related to the degree of chlorosis, but with two exceptions (treatments 1 A and 1 B) the Ni/Fe ratio is related. The result from Treatment 1 A is difficult to explain in view of the low level of nickel and the apparently satisfactory iron content, but with treatment 1 B the low level of total-iron may itself have been responsible for the high degree of chlorosis. The reduction in degree of chlorosis is therefore dependent on an increase in iron content relative to nickel, and as such is apparently linked to an increase in the availability of iron in the tissues; unfortunately determination of soluble fractions of iron, previously mentioned as useful measures of internal iron availability, was not possible in these experiments, but confirmation was obtained on analysis of material from the water-culture experiment.

TABLE 3. *Relation between nickel and iron contents and degree of chlorosis*

		Concentration (p.p.m.) in leaf dry matter		
Degree of chlorotic symptoms	Treatment no.	Ni	Fe	Ni/Fe
Very high	3 B	548	41	13·4
	3 A	637	63	10·1
	6 A	723	79	9·2
	6 B	480	59	8·1
	2 B	275	42	6·5
High	9 B	365	90	4·1
	2 A	226	62	3·6
	9 A	524	160	3·3
	1 A	6·3	64	0·98
	1 B	6·5	50	0·13
Low	5 B	105	59	1·8
Nil	5 A	147	88	1·7
	8 B	77	75	1·0
	8 A	127	144	0·88
	4 A	5·4	120	0·045
	4 B	4·0	95	0·042
	7 B	4·2	101	0·042
	7 A	3·9	147	0·027

Turning to the water-culture experiment, examination of the analytical figures for major nutrients in the no-nickel series indicated that the nutrient status of the plants was constant irrespective of pH, and yield (as estimated by height) differences were small. In this series the total iron figures decreased with increasing pH, but the lowest iron level in the plants was not associated with visual signs of iron deficiency.

In the nickel series the position was quite different. The nitrate and potassium contents remained constant (at about the same level as for the no-nickel plants). The phosphorus content, which could not be considered low in any treatment, increased where the pH was above 5, an increase in the presence of nickel which has been commented upon already. The plants as a whole did not present any marked abnormality in major nutrient status, although the potassium content was rather higher than normal and the calcium content rose substantially at higher pH values.

In the nickel series two types of symptoms developed, namely, a necrotic striping typical of nickel toxicity, and a marked diffuse chlorosis. At harvest, the necrotic condition was practically identical at all pH levels though the rate of development varied; the chlorotic condition, however, varied in intensity, being most severe at pH values over 6. Necrosis appeared first at the higher pH values while chlorosis appeared later, and then only at pH 5·5 and over. When leaves at various stages of chlorotic development were painted with a 0·5 % solution of ferrous sulphate, the chlorosis was replaced in most cases by a normal green colour. The necrosis, on the other hand, was neither removed nor prevented. This suggests that whereas the chlorotic condition was due to iron deficiency, the necrosis was a specific nickel effect. Nickel uptake was related directly to pH, although in view of the similarity in degree of necrotic symptoms on harvesting, the wide range in nickel contents (62–153 p.p.m.) is surprising—but probably accounts for differences in the rate of appearance of necrosis at the various pH levels.

TABLE 4. *Effect of* pH *on the absorption of nickel*

Treat-ment no.	pH of nutrient solution	Concentration (p.p.m.) in nutrient solution		Average heights (cm.)	P.p.m. in stem extract					P.p.m. in leaf dry matter		Degree of symptoms	
		Ni	Fe		NO_3	P	K	Ca	Mg	Ni	Fe	Chlorotic	Necrotic
118	4·0–4·5	0	1·2	12	279	26	340	17	9·4	6	132	O	O
119	4·5–5·0	0	1·2	13	298	20	320	16	9·3	—	124	O	O
120	5·0–5·5	0	1·2	14	295	20	340	17	9·6	—	111	O	O
121	5·5–6·0	0	1·2	14	335	21	330	17	9·8	—	106	O	O
122	6·0–6·5	0	1·2	14	310	22	360	18	8·8	—	94	O	O
123	6·5–7·0	0	1·2	14	301	22	360	18	9·5	—	85	O	O
124	7·0–7·5	0	1·2	14	301	23	330	17	9·0	—	98	O	O
125	4·0–4·5	1·2	1·2	14	279	24	362	16	9·4	62	91	O	M
126	4·5–5·0	1·2	1·2	14	295	27	376	20	10·0	82	156	O	M
127	5·0–5·5	1·2	1·2	14	289	29	380	22	11·0	81	100	O	M
128	5·5–6·0	1·2	1·2	12	282	37	380	23	11·0	72	71	L	M
129	6·0–6·5	1·2	1·2	12	289	38	394	24	11·0	104	62	H	M
130	6·5–7·0	1·2	1·2	13	276	38	368	25	12·0	150	54	H	M
131	7·0–7·5	1·2	1·2	13	307	37	380	27	12·0	153	70	H	M

Symptoms on scale: H=high, M=medium, L=low, O=healthy.

As no iron deficiency symptoms developed in the no-nickel plants, the iron deficiency was obviously related to the supply of nickel and not solely to the high pH values of the solutions in which the chlorotic plants were grown. At these high pH values the total-iron content of the leaves was reduced in both series, but in every pH range the iron content was smaller in the plants supplied with nickel (with one exception where contamination may have been responsible).

It was concluded from these results that the chlorosis was probably caused by the low iron content of the plants, the amount of iron absorbed being reduced by the combined effect of nickel antagonism and high pH. A simpler explanation, namely, that iron had been precipitated in the nickel solutions, was disproved on analysing the nickel and no-nickel solutions of highest pH when differences in iron content were found to be negligible.

The further possibility that an internal nickel-iron antagonism existed, was explored by determination of the water-soluble iron contents of four selected treatments:

	Nickel concentration p.p.m. in nutrient solution	Content (p.p.m.) in leaf dry matter		
Solution pH		Water-soluble Fe	Total Fe	Total Ni
4·0–4·5	0	37	132	6
4·0–4·5	1·2	49	91	62
6·0–6·5	0	34	94	6
6·0–6·5	1·2	14	62	104

It is clear, then, that while the total-iron content of the leaves is reduced by nickel, the greatest reduction is in the water-soluble fraction, and the existence of an internal nickel-iron antagonism is thus confirmed.

It may be that the reduced availability of iron within the tissues is related to their phosphorus content, which is higher in plants of the nickel series with pH over 5·0. The low degree of chlorosis found in plants of the nickel series at pH 5·5–6·0 does not necessarily disprove the existence of such a relationship, although a high degree of chlorosis was to have been expected in view of the relatively high phosphorus content of these plants; the mild chlorosis is probably accounted for by the low nickel content of the plants, i.e. by the relatively high Ni/Fe ratio.

When the critical treatments applied in both series of experiments were repeated, similar results were obtained.

DISCUSSION

These experiments show that a reciprocal relationship exists between nickel and iron contents of leaves—the nickel content being materially reduced by high concentrations of iron in the nutrient solution, and the iron content by nickel, the former relationship being the more pronounced. Nickel toxicity symptoms (both chlorotic and necrotic) were found to be decreased by high concentrations of iron in the nutrient solution, the reduction in degree of necrosis being related to reduced nickel content in the leaf blades, and that of chlorosis to reduced Ni/Fe ratio. It appears then that the general assumption that specific symptoms of heavy metal toxicities are unrelated to iron metabolism is correct. The indication of the relationship found by the authors (Vergnano & Hunter, 1952) in anatomical studies—namely the possibility of necrotic symptoms being the result of severe iron deficiency—was not substantiated, a high-level iron supply reducing the severity of the necrosis no more than could be accounted for by reduction in nickel content; moreover, other investigations (Hunter & Vergnano, 1953) suggest that the necrosis may be the result of nickel interference in copper metabolism. The experiments described here provide evidence that though the chlorosis produced by heavy metals is due mainly to reduced availability of iron in the tissues, the reduction in iron content of the leaf is also a factor to be considered; as already mentioned, Sideris (1950) reports reduction of iron content or translocation associated with manganese-induced chlorosis. Several theories have been advanced to explain non-utilization of iron in induced iron deficiency. The form and function of iron in the plant, as distinct from the iron deficiency relationship, have been discussed by several authors, and this aspect of the problem is well summarized by Lindner & Harley (1944).

Rogers & Shive (1932) report reduction in iron solubility due to relatively high sap pH, but Bennett (1945) states that the sap of iron-deficient leaves does not necessarily have a higher pH than that of normal leaves. Somers & Shive (1942) suggest that as the active iron is probably ferrous (Kliman, 1937; Thorne & Wallace, 1944; Hewitt, 1951), the mechanism by which manganese induces iron deficiency is the oxidation of ferrous iron to ferric and the precipitation of this as unavailable ferric phosphorus organic complex. The basis of this argument is that the oxidation-reduction potential of manganese ($Mn^{4+} \rightarrow Mn^{2+}$) is greater than that of iron, but Hewitt (1948*b*, *c*) points out that this theory breaks down when similar mechanisms are applied to other trace-element toxicities and, though more plausible if associated with the oxidation-reduction potentials of complex ions, still does not cover all the facts. In any case, Leeper (1944) emphasizes that the original hypothesis is fallacious because Mn^{4+} could not exist in tissues without causing irreparable damage.

Baudisch (1943) suggests that trace-element antagonism may be explained in the replacement of one trace element by another in an organic complex. Jacobson (1945) found iron to be associated with a chlorophyll protein complex. Sideris (1950) produces evidence that iron occurs in combination with a proteinaceous substance as an enzyme and is indirectly associated with chlorophyll, and Sideris & Young (1949) suggest that manganese-induced iron deficiency is due to replacement of iron by manganese in a chlorophyll precurser (cf. Hewitt (1951) who considers that replacement of magnesium by manganese may occur). It seems then that the mechanism most likely to be responsible for the internal reduction in iron availability is a replacement of iron in some organic complex essential to normal metabolism.

It was found in the above experiments that the phosphorus content of the stem extracts was higher the greater the degree of nickel toxicity, but that the relationship was constant only at each iron level of the solution. It is possible that this indicated accumulation of phosphorus in plants receiving nickel was related to the smaller size of these plants. Reduced phosphorus concentration in the leaves by increased iron supply was probably due to reduced availability of phosphorus in the solution.

A commonly reported feature of induced iron deficiency is the relatively high phosphorus content of the chlorotic leaves, a finding consistent with the theory put forward by Somers & Shive (1942) that iron is finally made unavailable by union with a phosphorus organic complex. Olsen (1935) has given evidence of this accumulation of phosphorus and of associated precipitation of iron in the leaf veins. Sideris & Krauss (1934), Chandler & Scarseth (1941), Walsh & Clarke (1945) and Millikan (1947) have found that iron deficiency is produced or intensified by high-level phosphate supply, and Hunter & Vergnano (unpublished) that the effect of nickel toxicity is increased; the work of Sideris & Krauss (1934) suggests, however, that excessive phosphorus in the solution gives iron deficiency only if the iron concentration in the nutrient solution is low. An actual reduction in the total-iron content of the leaves, when high-level phosphorus was supplied, was found by Chandler & Scarseth (1941).

Whilst some of these authors regard the phosphorus effect as occurring within the plant, Franco & Loomis (1947) claim that their water-culture experiments show the effect to be external and caused only by soluble phosphate at pH 6 and over. Moreover, Bennett (1945) points out that iron deficiency is not necessarily associated with a high concentration of phosphorus in leaves, and has shown that iron deficiency is not produced when the phosphorus content of the leaf is considerably increased by injection. He suggests, however, that the critical factor may be the rate of supply of iron relative to the rate of adsorption of iron on phosphoproteins; if this theory is correct, this rate of absorption would probably be greater the higher the concentration of phosphoproteins, and consequently any increase in phosphoprotein, such as might result from increased phosphorus supply, would lead to a reduction in availability of iron in the leaves, deficiency being the more quickly produced if the rate of iron supply were also low.

There is little doubt that excessive phosphorus at a relatively high pH reduces the external availability of iron and is thus conducive to iron deficiency by reducing the amount of iron absorbed. The effect of high phosphorus levels within the plant is more obscure, but it is probable that under certain conditions high-level phosphorus may facilitate a reduction in the internal availability of iron. The results of the experiments described here indicate that if the phosphorus status is related to nickel toxicity, it must be considered with other factors.

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STUDIES ON A CANKER DISEASE OF CYPRESSES IN EAST AFRICA, CAUSED BY *MONOCHAETIA UNICORNIS* (COOKE & ELLIS) SACC.

III. RESISTANCE AND SUSCEPTIBILITY OF SPECIES OF *CUPRESSUS* AND ALLIED GENERA

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Strains A and B of *Monochaetia unicornis* produced cankers following inoculation into *Cupressus arizonica*, *C. benthami*, *C. lusitanica*, *C. macrocarpa*, *C. sempervirens*, *C. torulosa*, *Cupressocyparis leylandii*, *Chamaecyparis lawsoniana*, *Juniperus procera* and *Thuja orientalis*. Strain D was parasitic only on *Juniperus procera*.

Monochaetia unicornis grew well on agar media prepared from bark and sapwood extracts of *Cupressus* species and *Juniperus procera* but only slowly on *Podocarpus gracilior* bark agar.

Test trees of five species of *Cupressus*, grown commercially in East Africa, were alternated with artificially inoculated trees of *C. macrocarpa* in field plots. The subsequent development of natural infections in trees of the test species confirmed field observations that *C. macrocarpa* was the most susceptible.

Plots (each of 340 trees) of the five species were inoculated with mycelium. In 4 months, the most extensive cankers had developed in *C. benthami*, and this species also had the largest number of active cankers and the most rapid height growth. When examined again 4 months later, *C. macrocarpa* had the most extensive cankers and 60% of them were still active as compared with 20% in *C. lusitanica* and 6% in *C. benthami*. Delayed inoculation following wounding confirmed these results and in the dry season the number of successful infections was always highest in *C. macrocarpa*.

Periderm was formed in the bark and sapwood of the *Cupressus* species in response to wounding, the process being slower in *C. macrocarpa* and *C. torulosa* than in other species. Secondary growth was most rapid in *C. macrocarpa*. It is suggested that rapid height growth coupled with rapid secondary growth in this host favour the growth of the pathogen rather than the host reaction, and *C. macrocarpa* is consequently very susceptible. During the rains the fungus develops as rapidly in species having a slower secondary growth rate but in the ensuing dry season such infections dry out and the cankers are occluded.

INTRODUCTION

In the first paper of this series (Jones, 1953), certain data were presented on the incidence of natural infections by *Monochaetia unicornis* in plantations of *Cupressus macrocarpa* and *C. lusitanica*, and on the recovery from canker disease of these hosts. The incidence of the disease was always higher in *C. macrocarpa* than in

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C. lusitanica and occlusion of cankers was more effective in checking the progress of the disease in the latter species.

The present paper is concerned with inoculation experiments on species of *Cupressus* and allied genera (but including *Podocarpus* and *Widdringtonia*) which are grown in East Africa, and with more detailed studies of those *Cupressus* species which are of commercial importance.

TABLE 1. *Inoculation of strains of* Monochaetia unicornis *into species of* Cupressus *and other coniferous genera*

Species	Inoculations		
	Strain A	Strain B	Strain D
Cupressus arizonica	+	+	–
C. benthami	+	+	–
C. lusitanica	+	+	–
C. macrocarpa	+	+	–
C. sempervirens	+	+	–
C. torulosa	+	+	–
Cupressocyparis leylandii (*Cupressus macrocarpa* × *C. nootkatensis*)	+	+	–
Chamaecyparis lawsoniana	+	+	–
Juniperus procera	+	+	+
Podocarpus gracilior	–	–	–
Thuja orientalis	+	+	–
Widdringtonia whytei	–	–	–

+ = canker or die-back produced; – = no infection.

INOCULATION OF SPECIES OF *CUPRESSUS* AND OTHER CONIFEROUS GENERA

Young trees, usually in the second year of growth, were inoculated by placing standard disks of mycelium into vertical slits, 1 cm. long, penetrating to the wood. The slits were sealed with Sellotape which was removed after 3 weeks, and the results of the inoculations recorded (Table 1). Strains A, B and D of *Monochaetia unicornis* (Jones, 1954) were used.

Strains A and B were found to have a wide host range, whereas strain D caused only a die-back and a limited canker lesion on *Juniperus procera*, the host from which it was isolated originally.

CULTURAL STUDIES WITH NATURAL MEDIA

In order to study further the host range of *Monochaetia unicornis*, the fungus was grown on agar media prepared from the bark of a number of host species. Previously, a positive correlation was demonstrated (Jones, 1954) between the mean length of cankers and the size of colonies on potato-dextrose agar.

A preliminary experiment was performed using sterile, filtered, cold-water extracts of macerated bark and sapwood tissue. However, these extracts supported only sparse mycelial growth and they were abandoned in favour of boiled extracts.

Tests showed that the bark extracts supported heavier growth than foliage extracts made up on the same basis.

Bark and sapwood tissues were stripped from branches or leaders of 2-year-old trees. Samples of 25 g. fresh weight were boiled in 100 ml. distilled water for 30 min., filtered through absorbent cotton-wool and the filtrate made up to volume. Plates were prepared containing 20 ml. and 1·5 % agar. Media were also prepared from dried bark using 15 g. samples made up in the same way.

TABLE 2. *Growth of* Monochaetia unicornis *on bark agars made up on fresh-weight and dry-weight formulae*

	Mean diameter of colony (mm.) after 7 days at 26·5° C.		Water content of bark and sapwood
Species	Fresh weight	Dry weight	(%)
Cupressus arizonica	13·00	9·83	65·4
C. benthami	13·66	10·00	68·5
C. lusitanica	13·25	9·66	70·2
C. macrocarpa	12·83	9·83	68·0
C. torulosa	14·25	10·66	66·8
Juniperus procera	15·41	11·83	60·4
Podocarpus gracilior	9·08	7·42	53·4
S.E. of means	1·53	2·13	

RM40 strain A was used in all inoculations.

Disks (2 mm.) of mycelium (Jones, 1954) of *M. unicornis* (RM 40 strain A) were used to inoculate six plates of each medium, and the plates were incubated at 26·5° C. Diameter measurements were made after 7 days and are shown in Table 2, together with the water content of the various bark tissues used. With agar made up on a fresh-weight or a dry-weight basis there was no significant difference between the growth of *M. unicornis* on the five *Cupressus* species, but there was a significant difference between the growth on agars of these species and on *Juniperus procera* (P=0·02–0·01) and on *Podocarpus gracilior* agars (P<0·001). With agar made up on a dry-weight basis, the differences were not so significant, and this was to be expected considering the different water contents of the barks. There was still, however, better growth on *Juniperus procera* and *Cupressus torulosa* so that the resistance to infection and development of cankers in these species cannot be the result of an unfavourable nutritional environment for the fungus. The sparse growth on *Podocarpus gracilior* agar shows that there is some adaptation to parasitism in *Monochaetia unicornis* and the fungus is not ubiquitous in its nutritional requirements.

NATURAL INFECTION IN FIELD PLOTS

Half-acre plots (340 trees) were planted in which trees of the test species were alternated with trees of *C. macrocarpa* so that each test tree was surrounded by four trees of *C. macrocarpa*. The outside rows of each plot were planted with

C. macrocarpa only. Similar plots were used by Wagener (1948) in studies on a canker disease of cypresses in California caused by *Coryneum cardinale*. After 1 year's growth, the *Cupressus macrocarpa* interplants and surrounding rows were inoculated with mycelium from mixed cultures of virulent collections of *Monochaetia unicornis*, strain A. The plots were established at two sites in Kenya, Hombe and Chehe (Jones, 1953), and a control plot of pure *Cupressus macrocarpa* was included, in which only the alternate trees were inoculated. The test species were *C. arizonica*, *C. benthami*, *C. lusitanica*, *C. macrocarpa* and *C. torulosa*.

In May 1952, the first recording was made of test trees which had become naturally infected by conidia liberated from inoculated cankers (Table 3). Only the Chehe plots were recorded and, owing to the Mau Mau disturbances, it has not been possible to make a further count of infected trees. However, the first results do show that *C. macrocarpa* is the most susceptible of the species tested, thus confirming observations on natural infections in plantations (Jones, 1953).

TABLE 3. *'Natural' infections by* Monochaetia unicornis *at Chehe, April* 1952. *Interplants inoculated December* 1950

Host species	No. of trees infected (max. possible 136)
Cupressus arizonica	4
C. benthami	3
C. lusitanica	7
C. macrocarpa	13
C. torulosa	1

EXTENSION OF CANKERS IN INOCULATED TREES

Half-acre plots of the five *Cupressus* species, 18-month-old trees (*C. lusitanica* 15 months old), were inoculated with mycelium of *Monochaetia unicornis* (strain A) under standard conditions, between 15 and 30 May 1951. The number of active cankers and their longitudinal extension were recorded in June 1951, and again in September 1951. The results are shown in Table 4, together with the mean height of twenty trees of each species as measured in June 1951. Measurements of the lengths of cankers gave an accurate picture of their extent with all species except *Cupressus arizonica*. In this species the fungus grew very rapidly up and down the stem of an inoculated tree, but the lateral extension of the canker was very limited and the area covered by the canker was, therefore, small. In subsequent experiments at Hombe, the cankers in *C. arizonica* were more similar to cankers in other species, but there was still the tendency in individual trees for the formation of long, narrow cankers. Considering the other species, *C. benthami* had the longest cankers, with *C. macrocarpa*, *C. lusitanica* and *C. torulosa* following in that order; this was also the order of height growth. The number of successful infections was low in *C. lusitanica* and *C. torulosa*. With the latter species this was expected, but with *C. lusitanica* the low figure may have been due to less favourable conditions for infection since this was the last plot to be infected and there was no rainfall after inoculation.

At the second recording, the number of active cankers and the longitudinal extension were much higher in *C. macrocarpa* than in any other species. A number of the *C. arizonica* and *C. lusitanica* infections remained active, but only 6 % of the *C. benthami* cankers were still growing and there were only two trees of *C. torulosa* with active cankers.

This experiment demonstrated that the host environment, as expressed in the rate of extension of cankers, was not the major factor determining the resistance of a host. Effective resistance to the spread of the fungus appeared to be dependent on a rapid reaction of the host tissues rather than on an unfavourable nutrient condition of the host for the pathogen.

TABLE 4. *Longitudinal extension of cankers in inoculated trees at Chehe. Half-acre plots of each species* (340 *trees*) *inoculated* 15–30 *May* 1951

Host species	Mean height (cm.)	Active cankers (%)		Mean length of canker (cm.)		No. trees killed
		Sept. 1951	Jan. 1952	Sept. 1951	Jan. 1952	
Cupressus arizonica	128·0	94·6	11·1	11·06	11·12	0
C. benthami	241·1	97·6	6·0	9·86	9·87	1
C. lusitanica	197·4	82·9	20·6	6·36	6·94	0
C. macrocarpa	212·7	92·8	63·2	9·06	11·86	11
C. torulosa	124·1	70·5	0·6	5·27	—	0

DELAYED INOCULATION OF WOUNDED TREES

One hundred and eighty trees (only eighty in the January experiment) of each of the five *Cupressus* species were given a standard wound 1 cm. high which penetrated to the heart wood of 18-month-old trees at half-height. Samples of twenty trees of each species were inoculated immediately with mycelium of *Monochaetia unicornis* (strain A) under standard conditions. Further samples of twenty trees were inoculated 2, 4, 6, 8, 10, 12, 14 and 16 days after wounding (2, 6 and 10 days in the January experiment).

Since a conidium or an ascospore are the normal agents of infection (Jones, 1953) it would have been preferable to have used spore inoculations in these experiments, but this method of infection could not be controlled adequately.

The experiment was carried out in November 1951, and repeated in January 1952. The number of successful inoculations and the longitudinal extension of cankers from immediate inoculations were recorded and are shown in Table 5A and B.

November 1951 (Table 5 I). In most instances, the longer the delay before inoculation, the smaller was the number of successful infections. At 6 and 14 days after wounding, however, the number of successful infections was much higher than would have been forecast, presumably due to heavy rainfall at the time of

TABLE 5.

A. *Numbers of active cankers developing from delayed inoculations after wounding, and numbers of cankers remaining active*

I. Hombe; November 1951

Time of inoculation (days after wounding)	Nos. of active cankers (max. possible 20)									
	C. ari.		*C. ben.*		*C. lus.*		*C. mac.*		*C. tor.*	
	Dec.	Apr.	Dec.	Apr.	Dec.	Apr.	Dec.	Apr.	Dec.	Apr.
Immediate	17	6	18	5	15	8	20	19	14	0
2	6	1	5	0	4	3	10	7	5	0
4	8	3	13	4	10	1	15	15	5	0
6	14	0	14	6	13	5	16	14	7	0
8	1	0	11	2	11	4	7	3	10	0
10	8	1	9	2	6	1	6	4	2	0
12	6	0	8	2	5	0	3	2	3	0
14	13	4	14	2	9	5	12	10	13	0
16	6	1	9	2	9	3	2	1	4	0
Totals	79	16	101	25	82	30	91	75	63	0

II. Hombe; January 1952

	Feb.	Apr.	Feb.	Apr.	Feb.	Apr.	Feb.	Apr.	Feb.	Apr.
Immediate	14	5	12	6	13	5	12	8	11	1
2	8	3	9	1	9	2	11	8	5	0
6	2	1	2	1	1	0	10	5	5	0
10	4	1	4	2	5	3	7	3	1	0
Totals	28	10	27	10	28	10	40	24	22	1

B. *Longitudinal extension of cankers developing from 'immediate' inoculation*

I. Hombe; November 1951

	Mean length of canker (mm.)				
	C. ari.	*C. ben.*	*C. lus.*	*C. mac.*	*C. tor.*
After 37 days	34·70	38·94	25·33	37·45	15·86
After 180 days	84·00	(95·75)	75·12	102·70	—

S.E. of species means (for 37 days only) = 2·35

Mean height of inoculated trees (cm.)	155·7	283·0	276·5	245·5	147·5

II. Hombe; January 1952

	C. ari.	*C. ben.*	*C. lus.*	*C. mac.*	*C. tor.*
After 37 days	17·79	21·83	21·38	17·75	14·91
After 108 days	(40·16)	52·00	(66·40)	51·33	—

S.E. of species means (for 37 days only) = 1·76

Mean height of inoculated trees (cm.)	158·5	282·5	325·0	257·5	166·0

The figures in brackets are the means of five readings only.

inoculation. The largest number of successful inoculations was in *Cupressus benthami*, with *C. macrocarpa*, *C. lusitanica*, *C. arizonica* and *C. torulosa* following in that order. There was no significant difference between the length of cankers on *C. benthami* and those on *C. macrocarpa*, but the length of *C. lusitanica* cankers was significantly less ($P=0{\cdot}01$–$0{\cdot}001$), and the difference between *C. lusitanica* and *C. torulosa* was highly significant ($P<0{\cdot}001$). The species with the most rapid height growth was *C. benthami* and, since the longest cankers were formed on this species, the data for height growth and length of canker were analysed to determine if there was a positive correlation. When the data from the five species were considered, the regression coefficient was found to be significant ($P=0{\cdot}05$–$0{\cdot}01$), but when the data for *C. benthami*, *C. macrocarpa* and *C. lusitanica* only were analysed, the correlation was not significant, and in the smaller cankers resulting in the January experiment the correlation was not significant even for all five species.

TABLE 6. *Changes in the water content of the bark and sapwood of 2- to 3-year-old* Cupressus lusitanica *trees during* 15 *months, at Hombe*

Date of determination	Water content (%)	Monthly rainfall (in.)
1950		
September	—	0·68
October	58·8	2·20
November	61·7	14·45
December	—	4·39
1951		
January	66·8	0·32
February	60·1	2·46
March	—	5·95
April	—	5·96
May	68·8	14·54
June	—	2·12
July	60·2	2·54
August	59·6	1·18
September	58·6	0·37
October	68·2	12·79
November	70·2	7·29
December	69·3	9·74

The trees were examined for active cankers again in April. At this recording over 80% of the *C. macrocarpa* cankers were still active as compared with 36% of *C. lusitanica*, 25% of *C. benthami*, and 20% of *C. arizonica* cankers. There were no active cankers in *C. torulosa*. The *C. macrocarpa* cankers were the longest.

January 1952 (Table 5 II). This experiment was carried out at the end of the rains when the water content of the bark and sapwood (Table 6), and presumably also the moisture available to the fungus, were decreasing. That this condition would favour the host reaction, rather than the growth of the fungus, is shown by the smaller numbers of successful infections in all species as compared with the results of the November experiment. Forty trees out of a possible eighty of *C. macrocarpa* had active cankers as compared with twenty-eight of *C. lusitanica*. There was no significant difference between the length of cankers on *C. benthami* and those on *C. lusitanica* or between cankers on *C. macrocarpa* and those on *C. arizonica*. There was, however, a significant difference ($P=0{\cdot}02$–$0{\cdot}01$) between the length of cankers on *C. lusitanica* and those on *C. macrocarpa* and between cankers on *C. torulosa* and those on the other species.

The difference between the means of canker lengths for the two experiments was highly significant ($P<0{\cdot}001$), and this was expected because of the limited extension of cankers under the drier conditions of January and February.

The inoculated trees were examined again in April when 60 % of the *C. macrocarpa* cankers were found to be still active as compared with 35 % of the *C. lusitanica* cankers. The mean length of canker was highest in *C. lusitanica*, but this is not surprising in view of the small number of readings on which the mean was determined.

These experiments again confirmed that the reaction of the host was the major factor controlling resistance to infection and spread of the fungus in the tree. In November, when there was heavy rainfall, the spread of the fungus was favoured rather than the host reaction. As a consequence, differences between the species were not as apparent as they were in January when the drier conditions and lower water content of the bark tissues favoured the host reaction. The dry season in East Africa corresponds to winter in temperate zones in that tree growth is restricted and concentrations of mobile food substances are low, although water loss may be high. These conditions limit the development of canker infections.

WOUND HEALING IN *CUPRESSUS* SPECIES

Since there was clearly a correlation between the time that had elapsed after wounding and the number of successful inoculations, a more detailed study was made of the process of wound periderm formation in the five *Cupressus* species. Two-year-old trees of these species were given a standardized wound, and samples of bark and sapwood were cut from the edges of the wound 4, 12, 16 and 24 days after wounding. Longitudinal and transverse sections, 15–20 μ thick, were cut by hand and mounted in lactophenol. They were examined for the presence of wound periderm cells and the results are shown in Table 7. Suberization commenced very soon after wounding and penetrated as deep as six layers of cells in all species except *C. torulosa* where suberization was very limited. The suberization was more extensive at the outer limits of the wound than in the new secondary bark and wood. Initiation of a wound periderm was apparent in a number of species 12 days after wounding, although it was delayed in *C. macrocarpa* and *C. torulosa* until 24 days. Nevertheless, the distinction between *C. macrocarpa* and *C. benthami* was very slight and did not appear sufficient to explain the difference in resistance to infection between these two species. However, *C. macrocarpa* had a thicker bark than the other species and it was apparent that the inner tissues of a thick-barked species were slow in forming a wound periderm.

TABLE 7. *Formation of periderm by* Cupressus *species in response to wounding*

Species	Time after wounding (days)	Nos. of layers of periderm cells Old tissue	 New secondary tissue
Cupressus arizonica	4	0	0
	12	0–1	0
	16	2	0
	24	4–5	2–3
C. benthami	4	0	0
	12	0	0
	16	1	0
	24	3–4	2–3
C. lusitanica	4	0	0
	12	0–1	0
	16	2	0
	24	4–5	2–3
C. macrocarpa	4	0	0
	12	0	0
	16	0	0
	24	3–4	2
C. torulosa	4	0	0
	12	0	0
	16	0	0
	24	1–2	1

TABLE 8. *The relation of height growth to secondary thickening in* Cupressus *species*

Species	Mean height (cm.)	Secondary wood at ½ height (mm.)	Bark and sapwood (mm.)	Ratio: bark/secondary wood
Cupressus arizonica	200	18·0	1·379	0·0766
C. benthami	372	24·0	1·268	0·0524
C. lusitanica	379	24·5	1·366	0·0557
C. macrocarpa	364	30·3	1·698	0·0560
C. torulosa	185	12·0	1·414	0·1178

The height, thickness of secondary wood, and thickness of bark and sapwood at half-height were measured in ten 2-year-old trees of each of the five *Cupressus* species (Table 8). The bark tissues were thickest in *C. macrocarpa* and the ratio of bark to secondary wood was higher in this species than in either *C. benthami* or *C. lusitanica*, the two species with similar height-growth.

DISCUSSION

Although no histological studies of the growth of the fungal mycelium in infected bark tissues have been made, it does appear that the species with the thickest bark —the species with the most rapid secondary growth—is also the species most susceptible to infection and to development of cankers. Under conditions of heavy rainfall, such as were experienced in the November experiment, with delayed inoculation following wounding, the difference between the 'thin-bark' and 'thick-bark' species is not so apparent, since the fungal mycelium can spread rapidly in the surface tissues. In the dry season, infections in the 'thin-bark' species tend to dry out, and only in thick bark can the fungus persist without desiccation or limitation by the host reaction. This applies to species which have approximately the same height-growth rate. In a slower growing species, such as *C. torulosa*, even though the bark is thick and the host reaction slow, there is not a sufficient supply of mobile food substances passing to the infected zone to enable the fungus to grow rapidly. As a consequence, only small cankers are formed and they are soon stopped by the host reaction.

Wagener (1948) observed over a number of years that *C. macrocarpa* was very susceptible to *Coryneum* canker, that *Cupressus lusitanica* was moderately susceptible and that *C. arizonica* was not susceptible. These results are similar to those obtained with *Monochaetia*, except for *Cupressus arizonica*. Wagener noted also that, when a plantation was thinned, cankers on trees left standing tended to develop more rapidly. A similar observation was made in Kenya, although no measurements were made to substantiate it. These observations, coupled with the evidence presented above, indicate that the fungus spreads most rapidly in species with a rapid height-growth. However, the fungus can only persist in an active, or potentially active, state when a rapid height-growth is coupled with a rapid secondary-growth, and it is only in *C. macrocarpa* that these requirements are satisfied.

In terms of control of the disease, it is now policy to remove all *C. macrocarpa* trees in diseased plantation areas. This has already led to a large drop in the incidence of infections in the neighbouring *C. lusitanica* plantations of one area (Jones, 1953).

The effective parasitism of *Monochaetia unicornis* appears to depend on the rate at which the fungus can grow in the living tissues of the host, and this is balanced against the reaction of the host tissues to invasion by fungal hyphae. There is a danger that a new strain of the pathogen may evolve which has a faster growth rate than existing collections of strain A. Such a strain might prove to be an effective parasite on *Cupressus lusitanica*.

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BREAKDOWN OF TIMBER BY ASCOMYCETES AND FUNGI IMPERFECTI

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(With Plate 11)

Soft rot of wood is caused by certain species of Ascomycetes and Fungi Imperfecti. The fungal hyphae typically run spirally in the central zone of the secondary xylem walls, where they give rise to cavities with pointed ends.

The fungi causing soft rot are cellulose-attacking species which initiate attack in the less heavily lignified parts of the walls of the wood elements. The rate of attack is increased when inorganic nutrient salts are added to the wood.

Under laboratory conditions *Chaetomium globosum* can cause severe decay of hardwoods.

Soft rot causes serious deterioration of timber in water-cooling towers and is therefore of considerable economic importance.

I. The type of attack and its distribution

Investigations on wood-destroying fungi have dealt mainly with Basidiomycetes and a few of the larger Ascomycetes such as *Daldinia* and *Xylaria* spp. The present study of certain Ascomycetes and Fungi Imperfecti arose from an investigation of surface deterioration of timber in water-cooling towers.

Previous work

Bailey & Vestal (1937) found hyphae of fungi, thought to be Pyrenomycetes, in the central layer of the secondary wall of wood elements of 114 species from thirty-six families of Gymnosperms and Angiosperms. The hyphae lay in cavities with pointed ends, and such cavities often occurred in chains. Tamblyn (1937) noted similar attacks on jarrah (*Eucalyptus marginata*) and found that the cavities were connected by extremely fine hyphae.

Barghoorn & Linder (1944) isolated Fungi Imperfecti and Pyrenomycetes from wood blocks and cordage exposed in the sea. Infected timber showed hyphae of the 'sap-stain type' and also colourless hyphae attacking the wood as described by Bailey & Vestal and causing a surface decay. Many of the fungi strongly attacked cellulose.

Findlay & Savory (1950) briefly noted similar attacks of wood; a more detailed account of these attacks is included below.

Appearance of decayed wood

The term soft rot is proposed for decay caused by cellulose-destroying micro-fungi to distinguish it from the brown and white rots caused by the wood-destroying Basidiomycetes.

Soft rot is usually superficial. Deep penetration occurs only in wood which is not continually waterlogged, and even then the severity of decay progressively decreases below the surface. Dull brown or blue grey discoloration, failure of the wood with a brittle fracture and softening of the surface so that it can easily be dug away with the point of a penknife are the usual indications of decay. Secondary insect attack is often present in timbers from buildings.

The surface of decayed submerged wood is often very soft and may be eroded. Softwoods usually show marked surface darkening and a rapid transition from decayed wood to a sound core. As submerged wood dries, its surface cracks across as in lightly charred wood; below lies a thin light-brown layer of less decayed wood overlying the sound wood of the core.

Microscopic examination

Sections were cut on a sledge microtome. Severely attacked hardwoods were strengthened by immersing blocks in molten paraffin wax and impregnating *in vacuo*. The stains chiefly used were Cartwright's (1929) safranin-picro-aniline blue and Gram and Jørgensen's (1953) safranin fast-green.

Softwoods

Irrespective of the species of timber, longitudinal sections from submerged timber usually show the following zones:

(*a*) *The central core.* This is composed of sound wood often containing sap-stain fungus hyphae, some of which stain in picro-aniline blue (a characteristic of young hyphae). Mature fungus hyphae are usually present in the other zones also.

(*b*) *Zone of attack.* The wood appears sound, but a few hyphae which have taken up the stain grow spirally within the tracheid walls of the summer wood, where there are chains of cavities with pointed ends as described by Bailey & Vestal (Pl. 11, fig. 2). Particularly in the earlier summer wood these cavities are elongated and contain thin-walled non-staining hyphae. The cavities all lie in the central part of the secondary walls of the tracheids (Pl. 11, fig. 3). Passing outwards there is increasing attack of the tracheid walls of the summer wood; numerous empty septate hyphae lie in the walls and some hyphae may have thickened walls. Transverse sections show that the cavities have greatly increased in size. In the walls of the spring wood there are fine hyphae growing spirally which lie in very narrow cavities (Pl. 11, fig. 1). In the lumina of both spring and summer tracheids there are often many other hyphae and occasionally chlamydospores, small pycnidia and bacteria.

(*c*) *The outer zone.* This is usually sharply differentiated; the wood is severely decayed and breaks away easily. Dissolution of the central layer of the secondary wall of summer tracheids is almost complete (Pl. 11, fig. 6). Fungal hyphae are usually not evident; sometimes they appear to have coalesced with the innermost layer of the secondary wall to form resinous masses. Towards the exterior the rays appear to be more resistant than the wood, but no details can be discerned.

Hardwoods

Visible attack is confined to fibres and, to a smaller extent, the rays. Different zones are not so clearly distinguishable but otherwise the attack is similar to that found in softwoods (Pl. 11, fig. 5). The fungal hyphae within the wall often appear to lie almost parallel to the long axis of the wood element; among the softwoods this has been noted only in Douglas fir.

Situations in which soft rot has been observed

A list of situations from which soft rot has been recorded is given in Table 1. It is of general occurrence in waterlogged wood but has also been noted in fence posts, logs and sawn timber.

Soft rot has been observed in eight species of softwoods and nineteen species of hardwoods and, in both, attack has been found in the heartwood of timbers known to be very resistant to fungal attack. Severe decay of sawn timber has been found in perishable hardwoods from the tropics.

Isolation of fungi

Chips or small blocks of wood were plated on to 5 % malt agar, 5 % malt agar with 0·5 % malic acid, prune-extract agar, potato-dextrose agar and a synthetic medium proposed by Abrams (1948) containing 3 g. NH_4NO_3, 2 g. K_2HPO_4, 2·5 g. KH_2PO_4 and 2 g. $MgSO_4.7H_2O$ per litre of tap water. A medium incorporating 0·2 % KNO_3, 0·2 % KI and 1 % of NaCl and glucose was useful for isolating marine fungi.

The species of fungi isolated are listed in Table 2. Isolates for which the herbarium number of the Commonwealth Mycological Institute is given (e.g. IMI 46257) have been identified at C.M.I.

One true marine species, *Helicoma maritimum*, has been isolated, but from wood immersed in fresh water terrestrial species only have been obtained. *Chaetomium globosum* was the fungus isolated most frequently. Fungi known to cause sap stain are recorded at the foot of Table 2.

Inoculation experiments

Methods normally used for exposing timber to attack by wood-destroying fungi were employed without success. No attack was obtained on softwood blocks which

TABLE 1. *Examples of soft rot which have been verified by microscopic examination*

Situation	Softwoods	Hardwoods	Plywoods
Water-cooling tower slats	*Scots pine (12, 7†) Douglas fir (1) Californian redwood (1) Pitch pine (2) Silver fir (1) *Spruce (1) Western red cedar (1)	*_Dipterocarpus_ sp. (1)	—
Atmosphere condenser	Scots pine (1)	—	—
In fresh water:			
Piling	Scots pine (1†, 1)	—	—
Boats	—	Gaboon (2) Mahogany (1)	Birch and gaboon (1) Birch and maple (1) Elm and other timbers (1)
Water-wheel	—	Oak (1)	—
Swimming-bath cubicle	—	*Teak (1)	—
In sea water:			
Piling	—	Greenheart (1)	—
Test samples—12 months immersion	—	Makoré (1)	*Gaboon (1)
Boat	—	—	Douglas fir (1)
Industrial vat	Scots pine (1)	—	—
In contact with soil	Yew (1) Scots pine (2)	Poplar (1†) *Saligna (1)	*Beech (1†) Gaboon (1)
Buildings:			
Floorboards	Scots pine (3)	—	—
Guttering	Scots pine (1)	—	—
Joists	Scots pine (6)	Oak (2)	—
Kitchen cupboard	—	—	Birch and gaboon (1)
Humidity room	—	*Beech (1)	—
Greenhouse	—	Teak (1)	—
Vehicles	—	Beech (1) Birch (1) Oak (1)	*Birch (1)
Imported timbers which had not been in service	—	Beech (1) *Chilean laurel (1) Coigue (1) Japanese oak (1) Krabak (1) Yang (1)	—
Exposed in the open:			
Notice board	—	—	Birch (1)
Test pieces in tropics	—	—	Beech (1)

* Timbers from which isolations of fungi have been made.
† Preservative-treated timbers.

The figures in brackets indicate the number of records for each timber.

had been previously autoclaved at pressures up to 40 lb. to cause partial decomposition of the wood. Multiple inoculation with several fungi was also without effect.

TABLE 2. *Fungi isolated from timber showing soft rot*

Name	No. of isolations	Isolate number if retained in culture
Ascomycetes:		
Chaetomium cochlioides Palliser	4	IMI. 46257, S. 109 B
C. elatum Kunze & Schmidt	2	IMI. 43663
C. funicola Cooke	1	IMI. 48590
C. globosum Kunze	Many	IMI. 33555
Orbicula parietina (Schr. ex Fr.) Hughes	1	IMI. 33761
Fungi imperfecti:		
Helicoma maritimum Linder	1	S. 130
Phoma glomerata (Corda) Wollenw. & Hochapf.	1	IMI. 46259
Trichoderma viride Pers. ex Fr.	Many	S. 596
Trichurus terrophilus, Swift & Povah	1	IMI. 46251
Incompletely named species:		
Alternaria sp.	1	S. 661
Aspergillus sp.	2	S. 640
Coniothyrium sp.	1	IMI. 46260
Coniothyrium sp.?	1	IMI. 46256
Cordana sp.	1	IMI. 46261
Fusarium sp.	Many	S. 586, S. 597, S. 600, S. 619
Hyalopus sp.?	1	S. 616
Hypomyces sp.?	2	IMI. 46250, 46254
Melanospora sp.	1	IMI. 41144
'Mucoraceous sp.'	Many	—
Periconia sp.	1	IMI. 46255
Penicillium sp.	Many	S. 639
Pestalozzia sp.	1	S. 530
Phoma sp.	3	S. 546, S. 610
Phoma sp. (Marine species?)	1	S. 653
Stemphylium sp.?	2	S. 618, S. 645
Stysanus sp.	1	S. 91
Type producing '*libertella*' conidia	1	S. 548
Mycelia sterilia	Many	S. 621, S. 624, S. 643, S. 644, S. 654
Fungi known to cause sap-stain:		
Cytospora sp.	1	—
Graphium sp.	1	—
Paecilomyces varioti Bain	Many	—
Phialophora sp.	3	S. 642
Pullularia pullulans (de Bary & Löw) Berkh.	4	—

Isolate numbers prefixed S are reference numbers of the F.P.R.L. culture collection.

Isolate numbers prefixed IMI are herbarium numbers of the Commonwealth Mycological Institute.

Soft rot was found in the upper half of blocks of avodiré, *Turraeanthus africanus*, exposed to attack by wood-destroying Basidiomycetes but colonized by *Chaetomium globosum* on their upper portion. Ultimately soft rot of beech was obtained with *C. globosum* using methods devised for testing fabrics (Abrams, 1948) by planting beech blocks on Abrams's medium, inoculating them with a spore suspension and incubating at 30° C. Sterilization of the beech blocks was unnecessary. In all experiments perithecia developed on the wood after about 5 days and formed a compact mat on the wood and spread thinly on to the agar after 6 weeks' incubation. Fig. 4 shows attacked beech as seen under the microscope.

On similarly treated softwood blocks thin mycelial growths developed with but few perithecia and there was no measurable decay of the timber. Although attack with *C. globosum* visible under the microscope was consistently obtained in softwood sections or softwood blocks on Abrams's agar after 14 days' incubation at 30° C., attack comparable with that on beech has not been obtained even after much longer periods of incubation.

The following weight losses were obtained when beech blocks, impregnated with solutions of nutrient salts, were exposed to attack by *C. globosum* for 6 weeks at 30° C.

	Average weight loss % (6 blocks)
K_2SO_4	Negligible
$MgSO_47H_2O$	Negligible
NH_4Cl	2·4
$(NH_4)_2HPO_3$	3·5
NH_4NO_3	3·2
$(NH_4)_2SO_4$	4·3
Abrams's solution	7·7

C. globosum can attack beech blocks on Abrams's agar at temperatures from 20 to 32° C. but not at 38° C.

Results of other experiments are summarized in Tables 3 and 4.

Discussion

True aquatic fungi can cause soft rot of timbers immersed in the sea but apparently only terrestrial species rot timber in fresh water.

Findlay & Savory suggested that in water-cooling towers partial hydrolysis of timber by heat or by attack of Basidiomycetes might be necessary before soft rot could occur. It has now been shown that a number of fungi can cause soft rot in sound timber.

The cellulose micellae of the central layer of the secondary wall of tracheids are orientated in a spiral of steep pitch as opposed to the shallow pitch of orientation in the other two layers (Clarke, 1938) and Bailey & Vestal have shown that the path of the fungal hyphae closely corresponds with the orientation of the micellae. It is generally agreed that the primary wall and first layer of the secondary wall are heavily lignified but that the central layer of the secondary wall contains little or no lignin. The apparent restriction of attack to the central layer suggests that initially attack by the soft rot fungi is inversely proportional to the amount of lignification of the cellulose.

Species of *Chaetomium*, such as *C. globosum* and *C. cochlioides*, are already known as destroyers of cellulose fabrics and it is possible that many of the more active cellulose destroyers may also be able to cause soft rot in timber.

TABLE 3. *Fungi proved capable of causing soft rot in pure culture*

Name	Timber used	Average wt. loss (%)	Method of exposure of timber	
Stysanus sp.	Pine sapwood	—	Sections embedded in Abrams's agar	
Chaetomium globosum	Pine sapwood	—	Sections embedded in Abrams's agar	
C. globosum	Sitka spruce	—	Blocks impregnated with Abrams's solution	
C. globosum	Beech	2	Blocks on tap-water agar	6 weeks at 30° C.
C. globosum	Beech	17·9	Blocks $\frac{3}{16}$ in. thick*	
C. globosum	Beech	25·3	Blocks $\frac{1}{8}$ in. thick*	
C. globosum	Beech	32·8	Blocks $\frac{3}{16}$ in. thick†	
C. globosum	Beech	37·8	Blocks $\frac{1}{8}$ in. thick†	
C. elatum	Beech	21·9	On Abrams's agar	
C. cochlioides	Beech	18·6	On Abrams's agar	
Trichurus terrophilus	Beech	16·8	On Abrams's agar	
Coniothyrium sp.	Beech	—	On Abrams's agar	

* On Abrams's agar.

† Blocks impregnated with Abrams's solution then planted on Abrams's agar.

TABLE 4. *Results of laboratory infection experiments with* Chaetomium globosum

Timber	Botanical name	Degree of resistance to attack by wood-destroying Basidiomycetes	Average weight loss % during 6 weeks' exposure to attack by *C. globosum* using Abrams's mycelial mat method: Untreated	Leached in tap-water at 30–35° C.
Softwoods				
Scots pine sapwood	*Pinus sylvestris*	Not durable	0·6 (22)	Negligible (32)
Bahamas pitch pine	*P. caribaea*	Durable	1·6 (12)	0·9 (26)
Hardwoods				
Beech	*Fagus sylvatica*	Not durable	23·2 (12)	30·4 (12)
Gaboon plywood	*Aucoumea klaineana*	Not durable	19·6 (10)	18·8 (10)
Nyankom	*Tarrietia utilis*	Not durable	3·4 (4)	6·7 (8)
			22·8 (6)	28·6 (4)
Greenheart	*Ocotea rodiaei*	Very durable	Negligible (12)	Nil (12)
Opepe	*Sarcocephalus diderrichii*	Very durable	Negligible (12)	Nil (12)
Tallowwood	*Eucalyptus microcorys*	Very durable	3·3 (10)	4·5 (16)
Wallaba	*Eperua falcata*	Very durable	Negligible (12)	Nil (12)

Figures in brackets show the number of blocks from which the averages were taken.

In laboratory experiments (Table 4) extensive attack has been obtained with *C. globosum* on hardwoods but not on softwoods, probably because softwood cellulose is not so easily attacked by the fungus as it is protected by a higher proportion of lignin (Wise, 1944).

The addition of nitrogen as organic or inorganic salts increases the rate of decay of wood by Basidiomycetes (Findlay, 1934). Addition of nutrient salts increases the rate of decay by *C. globosum* to a greater degree and the extent of decay appears to be proportional to the amounts of nutrients available to the fungus; rates of decay equal to those obtained with the more active Basidiomycetes can then be obtained but in the absence of nutrients attack is very slow. It is probable that soft rot will be more severe when timber is in contact with soil or water containing greater amounts of nutrient salts. Nitrogen compounds are likely to be the most important of these.

The incidence of soft rot under natural conditions may often be explained by the fact that Ascomycetes and Fungi Imperfecti are tolerant of a wider range of conditions than the Basidiomycetes. Soft rot can thus be important in conditions that do not permit Basidiomycete attack yet do not completely inhibit fungal growth. Soft rot in waterlogged timbers reflects an oxygen supply too low for the Basidiomycetes.

A high proportion of decay of plywood is caused by soft-rot fungi but this is probably related to the high surface/volume ratio or to the presence of nitrogenous glues.

II. Soft rot in cooling towers

Much of the soft rot recorded above is not of economic importance, but soft rot appears to be an important cause of deterioration of the filling timbers in water-cooling towers.

The timber filling of a large tower may cost £10,000–£20,000 to install. It is exposed under conditions suitable for attack by soft-rot fungi, but as there are other factors which cause deterioration the relative importance of the different types of timber breakdown has to be assessed.

The water-cooling tower

A natural-draught cooling tower usually consists of a chimney of wood or concrete, in the base of which are placed banks of softwood slats arranged to disperse evenly a stream of warm water which is poured over them. The chimney causes an updraught of air which cools the water. The water usually enters the tower at a temperature of 35° C. and leaves at about 25° C. Most towers are continuously in use except during servicing periods.

Some circulating water may be run off to limit increases in salt content. This and any other loss is made good by the introduction of more water from any convenient source. Such 'make-up' water may be softened to prevent scale formation. In some towers the circulating water is occasionally chlorinated if diatoms and green algae accumulate on the slats sufficiently to impede uniform water distribution.

In some installations the whole volume of circulating water is drawn from a river and is passed through the tower to be cooled before being returned lower down the river.

Previous work on timber deterioration in cooling towers

Mechanical failure caused by loosening of nails, by ice formation, by heavy-scale formation or by severe erosion, has been recognized. Chemical attack on the wood can occur when the water becomes too acid through uptake of sulphur dioxide from the atmosphere or too alkaline due to injudicious softening (Baker, 1950). White and brown rots caused by Basidiomycetes occur in structural timbers (Skewes & Colwell, 1948) and in slats (Baechler & Richards, 1951). Soft rot has been recognized only in towers in Great Britain, but deterioration which resembles that caused by soft rot has been noted in Australia (Skewes & Colwell, 1948) and in the U.S.A. (Blohm & Butcher, 1952; Browning & Bublitz, 1953).

Observations on breakdown of timber in cooling towers

Five large cooling installations have been inspected, samples from about thirty other installations have been examined, and much information has been obtained from the British Electricity Authority who are also studying this problem.

Soft rot has been found in the surface of all cooling-tower slats examined including many which have received preservative treatment and some which have been in service for only 9 months. No soft rot was found in slats from a degassing tower after 11 years' service; fungal attack had probably been prevented by toxic chemicals present in the water.

Two examples of chemical attack of timber have been seen, but neither had necessitated any replacement of timber. Rot caused by Basidiomycetes occurs infrequently in the wooden chimney of older towers and in wooden troughing used to carry water within towers but has been noted once only in slats. Warping or other occasional failures of troughing lead to uneven distribution of water, loss of cooling efficiency and even severe erosion and breakage of slats on which large quantities of water fall. All slats become somewhat reduced in cross-section, but erosion is important only when sand and grit are present in the circulating water, for in many towers wood surfaces so softened that they can be removed by light pressure from the fingers still resist the impact of the falling water.

Soft rot always occurs on the inner surface of linings of wooden chimneys but in this situation it has not led to replacement of the wood in periods of up to 45 years of service. Serious soft rot of structural timbers has been noted only in a tower in intermittent use, but soft rot in hardwood dowels used to support banks of slats has led to premature failure of whole sections of a tower in which this type of construction was employed.

The service life of cooling-tower slats varies. Complete replacement after 3–5 years has been necessary, but 11 years is the expected life and up to 25 years service has been obtained.

Significance of the types of timber breakdown observed

Different types of timber breakdown often occur in the same tower and their effects are usually complementary. However, it is considered that soft rot is the primary cause of deterioration and loss of strength of slats in the great majority of cooling towers in this country; the excellent general condition of the slats unaffected by soft rot after service in the degassing tower supports this view.

On the other hand, soft rot is unimportant in structural timbers of large cross-section and Basidiomycete decay has greater significance. Such decay occurs surprisingly infrequently and is only likely to be found in timbers which are wetted infrequently or in towers which are little used.

Erosion is not of general significance but where it does occur its effects are increased by surface weakening of the wood by soft rot.

Factors affecting fungal attack in cooling towers

In laboratory experiments, the extent of attack by soft-rot fungi is roughly proportional to the amounts of nutrient salts present. Although the concentrations of nutrient salts in the circulating water are very much lower than those used in the laboratory, there are indications that a similar relationship with nitrates probably exists in the towers. This has particular significance because sewage effluent with high nutrient salt content is being increasingly used as make-up water and in other installations nitric acid has been used to reduce the alkalinity of the water.

Though soft rot may quickly weaken the surface of a submerged piece of wood, the depth of penetration is limited (apparently by the oxygen supply). It follows that a small increase in the area of cross-section in relation to surface area of the slats may give a much improved service life. The same result may be obtained if factors which tend to expose new surfaces to fungal attack are eliminated. Such factors are erosion by abrasive material in the water and drying during periods of disuse of the tower sufficiently to allow cross-cracking of the softened wood surfaces.

Timber treated by impregnation with wood preservatives is already in use in a number of towers, but as yet there are no results from controlled experiments to support claims that treated slats give longer service. Preservative treatment will undoubtedly prevent decay by Basidiomycetes in structural timbers which are wetted only occasionally, and should prevent decay of the slats themselves if the towers are not constantly in use. Treatment of the circulating water with toxic chemicals to prevent attack of the wood by fungi is too costly to be of value.

The timber from which the slats are made varies considerably in quality and it has been suggested that the better quality, denser, slow-grown softwoods have a longer life. Such timber is more resistant to erosion, but no indications have been found that it is less affected by soft rot than timber of poorer quality.

Laboratory experiments (Table 4) indicate that softwoods as a class are more resistant to soft rot than hardwoods, and it is significant that hardwoods are not found in use in towers. On the other hand, in the laboratory the most durable hardwoods have shown a high degree of resistance to soft-rot attack even after having been leached in warm water for 2–6 weeks. It is probable that initial cost has prevented any employment of these durable timbers in towers so the possibility remains that hardwoods of the highest natural durability may prove suitable for use.

I wish to thank the Director of the Commonwealth Mycological Institute for assistance received in identification of cultures.

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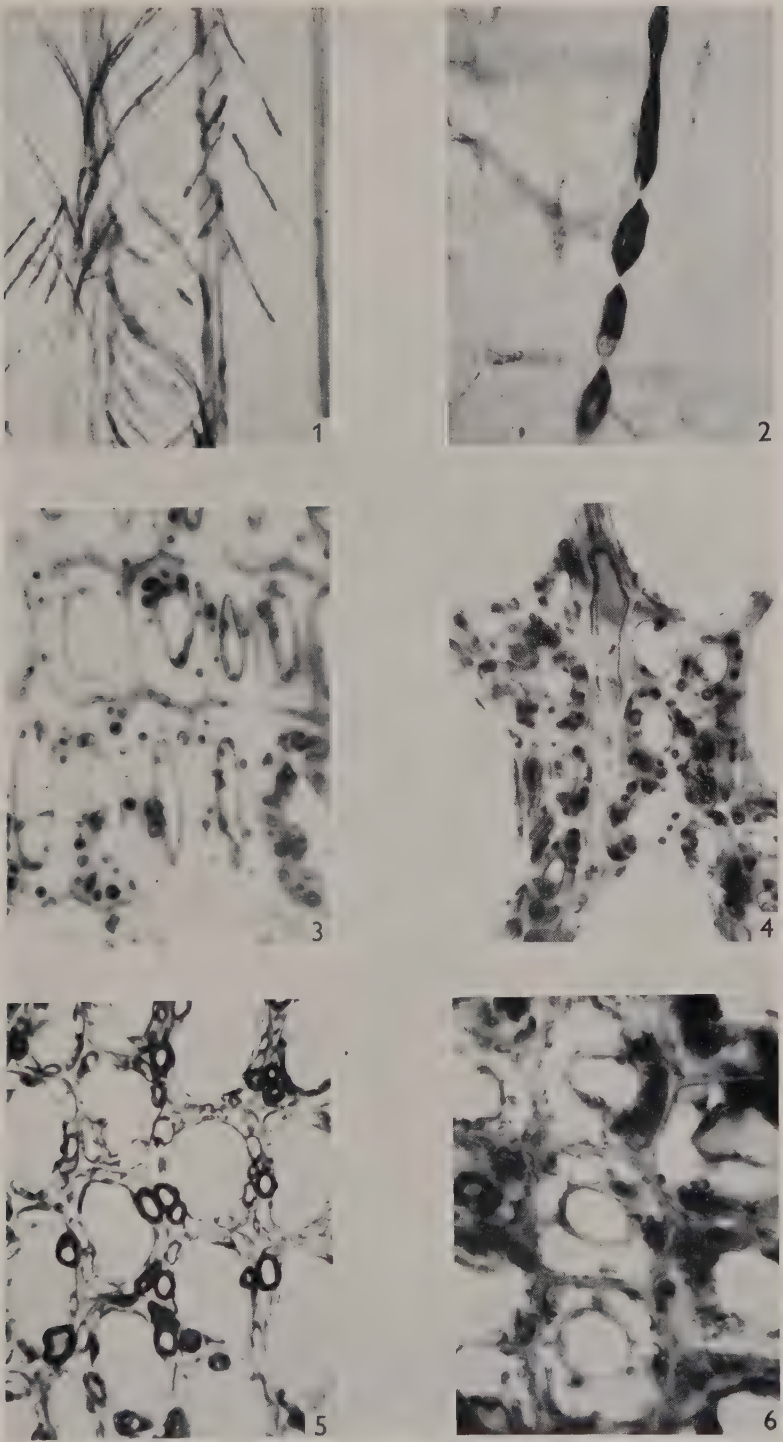

SAVORY—*Breakdown of timber by Ascomycetes and Fungi Imperfecti*

EXPLANATION OF PLATE 11

Fig. 1. Radial longitudinal section of springwood of pine showing the spiral course of the hyphae within the secondary wall (× 540).

Fig. 2. Radial longitudinal section of summer wood of pine from a wooden gutter. A chain of large cavities in the secondary wall (× 600).,

Fig. 3. Transverse section of Douglas fir summer wood from a water-cooling tower. An early stage of attack; the dark areas within the wall are fungal hyphae in cross-section (× 510).

Fig. 4. Transverse section of beech. Condition of fibres after 21 days attack by *C. globosum* in pure culture (× 510).

Fig. 5. Transverse section of Chilean laurel, *Laurelia aromatica.* A late stage of attack, in which most of the central layer of the wall has been decayed away. The dark circles in the wall are thick-walled fungal hyphae in cross-section (× 600).

Fig. 6. Transverse section of pine from a water-cooling tower. A late stage of attack. Dissolution of the central layer of the secondary wall has been almost completed (× 600).

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Meetings of the Association were held at the Imperial College of Science and Technology, London, on 8 January and 26 February 1954. The full list of speakers, with titles of papers, will be given in the Report of the Council of the Association.

The following are presented below, in slightly abridged form:

Paper read on 8 January

First steps in breeding for resistance to potato-root eelworm. By Mr F. G. W. Jones.

Papers read on 26 February

Fungal rots of apples, with special reference to *Gloeosporium* spp. By Dr E. H. Wilkinson.

The rabbit disease: myxomatosis. By Mr Harry V. Thompson.

FIRST STEPS IN BREEDING FOR RESISTANCE TO POTATO-ROOT EELWORM

By F. G. W. Jones, M.A., *School of Agriculture, University of Cambridge*

(With 2 Text-figures)

In 1938 an expedition went from Great Britain to collect tuber-forming species of *Solanum* from mountainous areas of south and central America, a region generally regarded as the cradle of potato species. The plants brought back by the expedition form the core of the Commonwealth Potato Collection, which has since been added to from various sources. In 1941 Dr Ellenby began screening the various species and varieties in the Collection for resistance to eelworm. Quite soon he was able to demonstrate resistance in *S. ballsii* (now known as *vernei*.) Later, after much labour and many disappointments, he also found evidence of resistance in a few lines of *S. andigenum*.

Breeding for resistance began at this point. The Dutch were first to take the initiative, and Dr Toxopeus and Ir Huijsman have been working at Wageningen and in the N.E. Polder for about 3 years with vigour and with excellent facilities. Work in Germany and Great Britain soon followed. In Germany, the plant breeding is carried out by Dr Ross at the Max Plank Institue, Voldagsen, Hann., and the nematology by Dr Goffart in Münster, Westf. In Scotland, work is in progress under Dr Black of the Scottish Plant Breeding Institute. In Cambridge, work is done jointly at the Plant Breeding Institute (Dr H. W. Howard) and the School of Agriculture (F. G. W. Jones and T. D. Williams) and is financed by the Agricultural Research Council. The Germans and the Dutch have divided the field between them, the Germans concentrating on breeding from *S. vernei* and the Dutch chiefly from *S. andigenum*.

Breeding from *S. vernei* is complicated by the fact that the plant is diploid whereas *S. tuberosum* is tetraploid. For this reason, when working with *S. vernei*, it is necessary first to obtain plants with double the chromosome number by colchicine treatment. Crossing is then easy, and satisfactory F_1 plants are produced, but in the F_2 the set of seed is poor. This is the stage reached in Germany at present and the various crosses are being tested for resistance.

Breeding from *S. andigenum* is simpler since the plant is tetraploid and is so close to the cultivated potato that it is now regarded as a mere variety of *S. tuberosum*. The Dutch and British work on *S. andigenum* is based mainly on Ellenby's resistant material from the Commonwealth Potato Collection (Table 1), and the first step taken in both cases was to confirm resistance. Closely similar results were obtained in Holland, England and Scotland using potato-root eelworm populations from three widely separated sources. Resistant seedlings were found in the ratio of about three to every one susceptible. It was at once clear that resistance was not complete, for on some of the resistant plants white or yellow female eelworms ('cysts') could be found in small numbers after a careful search, while susceptible plants bore cysts in great numbers.

TABLE 1. *Some breeding material used in Holland and Britain*

Lines of *S. andigenum* containing resisters (Holland and Britain)

H1 = C.P.C.	1692 selfed.	Cochabamba, Cercado, Bolivia
H2 = C.P.C.	1690 selfed.	Puno, Peru
H3 = C.P.C.	1685 selfed.	Juli, Puno, Peru
H4 = C.P.C.	1673 selfed.	La Paz, Bolivia

Susceptible *S. andigenum* (Britain)

H13 = C.P.C. 1787,
C.P.C. 1470

Lines of *S. vernei* (= *S. ballsii*) (Britain)

C.P.C. = 2487 } *S. vernei* selfed
C.P.C. = 2488 }

D3 = *S. stenotomum* × *S. vernei* F_2

C.P.C. = Commonwealth Potato Collection.

TABLE 2. *Ratios expected in tetraploid crosses when resistance is due to a single dominant factor* **H**

Type (1)	Symbols (2)	Nomenclature (3)	Gametes (4)	Back-crossed to susceptible (**O**) Resistant : suceptible (5)	Selfed Resistant : susceptible (6)
HHHH	HHHH	Quadruplex	All **HH**	All resistant (100 %)	All resistant
HHHh	HHH	Triplex	1 **HH** : 1 **Hh**	All resistant (100 %)	All resistant
HHhh	HH	Duplex	1 **HH** : 4 **Hh** : 1 **hh**	5 : 1 (83·3 %)	35 : 1
Hhhh	H	Simplex	1 **Hh** : 1 **hh**	1 : 1 (50 %)	3 : 1
hhhh	O	Nulliplex	All **hh**	All susceptible (0 %)	All susceptible

Other crosses

Simplex × duplex	11 : 1
Simplex × duplex	3 : 1

Before considering the ratios of resistant to susceptible plants obtained in various crosses it is necessary to consider briefly the complications arising from tetraploidy (Haldane, 1930). Assuming that resistance is due to a single dominant gene (**H**), then five types of plants are possible as shown in column (1) of Table 2. The usual symbols and the nomenclature adopted for these types are shown in columns (2) and (3), while the expected gametes and the results of crossing with the recessive (susceptible = **O**) or selfing are set out in columns (4), (5) and (6) respectively. Small deviations from these ratios would occur on the hypothesis of random segregation of chromatids rather than random segregation of chromosomes.

Deviations would be small for crosses with simplex and duplex plants and are chiefly of interest because they explain the appearance of susceptible plants in triplex crosses where only resisters are expected.

The results of the work by Toxopeus & Huijsman (1953), and our own preliminary work, are summarized in Table 3. Toxopeus & Huijsman interpret the results from breeding with plants of line H4 as fitting the theory that resistance is due to a single dominant gene and the ratios which they give are in fair agreement with this theory. Possible alternative ratios are shown in brackets, but more complicated genetic theories would have to be advanced to explain these. The crosses of line H3 with *S. tuberosum* gave a 1 : 3 ratio, suggesting that resistance might be due to the presence of two complementary factors designated **K** and **L**. Our own meagre results for crosses of H3 with *S. tuberosum* might be explained on the basis of either a single factor for dominance or of two complementary factors. The importance for breeding of the Dutch work with line H4, however, is not in the proof of any underlying genetic theory, but in the simple fact that resistance behaves as a dominant and gives rise to a high proportion of resistant plants when crossed with *S. tuberosum* varieties. The complementary type of resistance in line H3 if confirmed, would be less advantageous since it produces a lower proportion of resisters.

TABLE 3. *Ratios of resistant to susceptible plants in crosses of resistant* Solanum andigenum

Parentage	No. of seedlings		Type of cross	Expected ratio	Numbers of seedlings expected	
	Resistant	Susceptible			Resistant	Susceptible
			A. From Toxopeus & Huijsman (1953)			
H4×H4	70	4	**HH×HH**	35 : 1	72·0	2·0
				(15 : 1)*	(69·4)	(4·6)
	291	25	**HH×H**	11 : 1	289·3	26·3
	119	51	**H×H**	3 : 1	127·5	42·5
H4×*S. tuberosum* varieties	565	193	**HH×O**	5 : 1	631·5	126·3
				(3 : 1)*	(568·5)	(189·5)
	215	263	**H×O**	1 : 1	239·0	239·0
				(5 : 7)*	(199·2)	(278·8)
H3×*S. tuberosum* varieties	94	311	**KL×O**	1 : 3	101·2	303·7
Susceptible H4× *S. tuberosum*	0	129	**O×O**	0 : ∞	All susceptible	
			B. Tests made in Cambridge			
H3×*S. tuberosum* varieties	83	99	**H×O**	1 : 1	91	91
			KKL×O	5 : 7	75·8	106·2
H1×*S. tuberosum*	17	13	?	—	—	—
Susceptible H3× Susceptible C.P.C. 1470	0	50	**O×O**	0 : ∞	All susceptible	

* Possible alternative ratios in brackets.

Turning to the nature of resistance, it is possible to place plants in three arbitrary categories, namely:

(1) Absolutely resistant. Not invaded.

(2) Partially resistant. Invaded.
  (*a*) Larvae fail to develop.
  (*b*) Larvae develop but fail to mature.
  (*c*) Larvae mature but the females are reduced in numbers and not very prolific.

(3) Susceptible. Large numbers of highly prolific females produced.

Other considerations entering into the problem are whether or not the roots produce the hatching factor, whether or not the roots produce an additional and quite separate substance responsible for attracting larvae to them, and whether or not the roots respond to invasion by the production of giant cells. Work on larval invasion of resistant plants, on the production of the hatching factor and on the response of the roots to invasion has been started in Cambridge. The existence of a separate attractive substance is speculative at the present time.

TABLE 4. *Degree of invasion of the roots of resistant and susceptible* Solanums

Type	No. of plants tested	Stage 1 (%)	Stages 2 to 4 (%)	Males (%)	Small females (%)	Total all stages/g. root	Date counted
Susceptible							
Majestic	3	5·5	53·9	30·2	10·4	903	June
Gladstone	5	26·1	33·8	16·4	23·7	517	July
Resistant							
H 1	9	59·6	34·6	5·8	0	413	July
H 2	4	82·3	17·1	0·6	0	610	July
H 3	13	64·5	28·4	6·5	0*	349	July–Aug.
H 4	29	90·5	7·3	2·2	0	167	July–Aug.
Stenotomum ×	12	71·0	22·8	6·2	0	167	Mid-Aug.
vernei F_2	13	94·3	0	5·7	0	53	Late Aug.

* One small immature female observed on the roots of two plants in the samples under test.

Table 4 shows the results of investigations of the larval invasion of resistant plants. All the plants examined were invaded and produced subsequent stages in the proportions shown in columns (3) to (7). Generally the unmodified larvae as found in the soil (stage 1) made up the greatest percentage of forms observed. A small percentage of males which appeared normal were also noted, but there was a virtual absence of forms distinguishable as females, although in the root samples under test one small immature female was observed on the roots of each of two plants. The occasional appearance of a few cysts on resistant plants has already been noted, and is sometimes a source of embarrassment in the determination of the ratios of resistant to susceptible plants. Resistance is only partial (category (2*c*)) and this holds equally for the four lines of *S. andigenum*, for the crosses of *S. vernei* with *S. stenotomum* (the cultivated diploid potato), and for *S. vernei* itself. The final column in Table 4 gives the rate of invasion/g. of root. The falling off in the rate of invasion from the susceptible types at the head of the column to the *S. stenotomum* × *S. vernei* crosses at the foot is apparent rather than real and may be connected with the month in which the observations were made. The very low figures for *S. vernei* obtained in mid-August may possibly indicate somewhat greater resistance to invasion but requires confirmation. In experiments conducted upon the production of the hatching factor twenty-seven plants of *S. andigenum* drawn from the four lines (H 1, H 2, H 3 and H 4), all produced a root diffusate active to a greater or lesser degree, although the average was a little below the activity of *S. tuberosum* varieties Langworthy, Red Gladstone and Golden Wonder. *Stenotomum* × *vernei* crosses and *S. vernei* itself produced root diffusates of low activity, but there was some evidence for segregation of this factor in the former, one plant in twelve producing diffusate fully as active as that of *S. tuberosum*. In breeding for resistance it would obviously be an advantage to have two types of resistant plant, one producing little or no hatching factor which would tend to '*guard*' or enhance resistance and another type producing much of the hatching factor which would make the ideal trap crop after suitable breeding to give tubers of economic value, if only for feeding stock. The preliminary experiments outlined above suggest that the first type is more likely to be found by crossing with *S. vernei* than with *S. andigenum*.

The effect of resistant plants on soil populations has been investigated in pot experiments and the results are shown in Figs. 1 and 2. In Fig. 1, the effect of five individual resistant plants from each of the lines H 1, H 3 and H 4 are compared with fallow soil (O), susceptible *S. andigenum* and *S. tuberosum* seedlings (H 13 and H 14 respectively), and with plants grown from Gladstone tubers. At the start of the experiment 10 in. pots were filled from a mixed bulk of infested soil and sunk into the ground. At the end of the experiment the cyst counts (not 'viable' cysts) from the pots containing resistant plants showed no increase over the unplanted controls (O), whereas the susceptible plants caused increase in varying degrees. The cyst contents were investigated by a squash technique and there was evidence of a decrease in egg and larval counts for the pots containing resistant plants, and large but variable increases for the pots containing susceptible plants. Further batches of cysts were submitted to the action of a standard root diffusate from *S. tuberosum* and produced the hatching curves shown in Fig. 2. Although the hatch was not taken to completion, marked differences in the 'hatchable' contents of the different batches of cysts are apparent. It seems clear that the resistant plants produced few or no new cysts and that their active root diffusates caused partial emptying of the cysts already present.

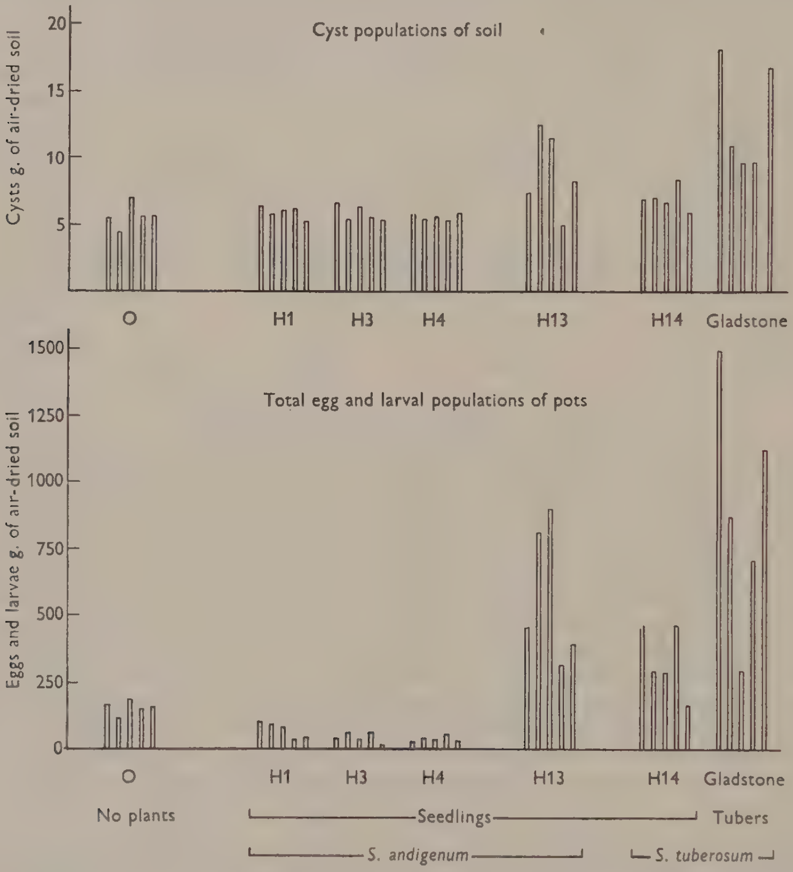

Fig. 1. The effect of susceptible and resistant potatoes on the eelworm population of the soil: results of pot experiment made in 1952.

According to Toxopeus & Huijsman (1953) the chances of establishing a new variety of potato in Holland from a random potato seedling are 1 in 50,000. The chances of establishing a new variety with resistance to eelworm and possessing all the other commercially desirable properties from *tuberosum* × *andigenum* crosses are much smaller. It will take some years and much labour before this goal is achieved.

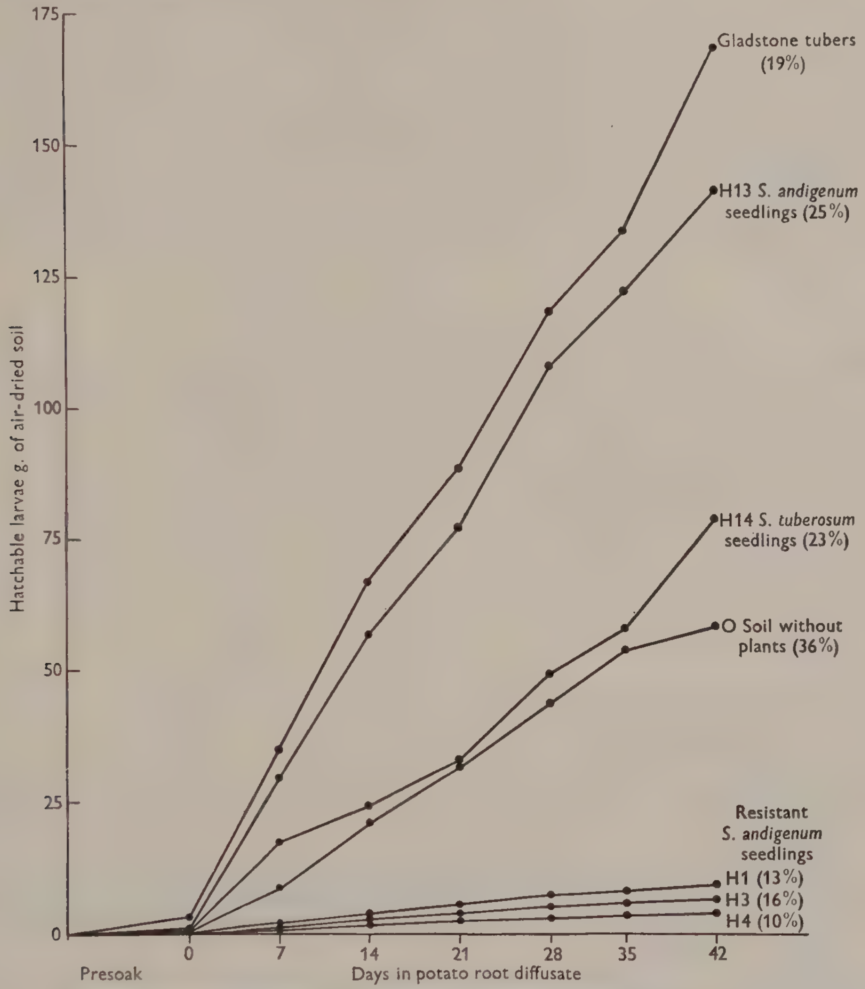

Fig. 2. The effect of susceptible and resistant potatoes on the cyst contents of soils from the 1952 pot experiment. The percentages at the right of the graphs are the proportions of cyst contents hatched in 42 days. The curves show the cumulative hatches of larvae and the points on them are means of samples from the five replicate pots.

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FUNGAL ROTS OF APPLES WITH SPECIAL REFERENCE TO *GLOEOSPORIUM* SPP.

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The storage of fruit under artificial conditions has increased considerably during the past thirty years despite the high capital charges and expense required for the erection and maintenance of the storage chambers. In 1925 there was storage space for 2570 tons of apples, whereas in 1950 this had increased to space for approximately 85,000 tons, consisting mainly of refrigerated gas-storage installations. This total is equivalent to 5,000,000 bushels and does not include the large quantities of fruit stored under 'barn' conditions or the more recently introduced 'moss' storage system.

Apples, like other natural products, when held in storage suffer from certain losses, the main causes being:

(1) Functional or non-parasitic breakdowns.
(2) Rots caused by various fungal pathogens.

Table 1. *Type and frequency of fungal rots recorded in cold stored apples during 1937–9*

Fungus	Percentage rots 1937–8	Percentage rots 1938–9
Gloeosporium spp.	15·41	8·16
Botrytis cinerea	6·56	8·08
Penicillium expansum	3·31	7·42
Sclerotinia fructigena	0·54	1·51
Alternaria spp.	0·23	0·04
Cylindrocarpon mali	0·20	0·79
Rhizopus nigricans	0·04	0·15
Diaporthe perniciosa	0·02	0·04
Fusarium spp.	0·01	0·07

	Season 1937–8	Season 1938–9
Total number apples stored	21,600	12,822
Number of rotted fruits	5,691	3,370
Percentage rotted fruits	26·32	26·26

Functional breakdowns are responsible for serious losses in some seasons and these have been investigated fully by Wallace (1953), especially in relation to the orchard factors which may be responsible for their incidence in store.

Before the second World War, the main fungal species causing storage rots had been identified and studied by Horne (1924), Kidd & Beaumont (1924), Colhoun (1938) and Ogilvie (1935), but no quantitative data were available concerning the relative importance of the apple-rotting species.

This information was obtained by the writer (Wilkinson, 1939, 1944*b*) during the storage periods 1937–8 and 1938–9 when apples collected from the main fruit-growing areas of England were placed in cold storage at Long Ashton. The results of these investigations are given in Table 1.

Three points of interest emerged from this work:

(1) The importance of the *Gloeosporium* spp. as rot-producing organisms.

(2) The high losses sustained, individually or collectively, by the attack of *Botrytis cinerea*, *Penicillium expansum* and *Sclerotinia fructigena*.

(3) The mode of entry of the various pathogens.

With few exceptions the rots caused by *Botrytis cinerea*, *Penicillium expansum* and *Sclerotinia fructigena* were the result of penetration via wounds. Thus, from the practical point of view, the reduction of these losses is largely in the grower's hands in the more efficient execution of his spray programme and the training of his workers to handle the fruit with the utmost care.

The invasion of the apple tissues by the *Gloeosporium* spp. is a different problem, however, because the main point of entry is through the lenticels. Hence, if it were possible to store a load of apples completely free from skin injuries there is no guarantee that after 4 or 5 months' storage these apples could be unloaded free from blemish. In fact, the experience of the past few years has shown that very serious losses, as the result of lenticel invasion, have occurred in Kent.

FUNGI CAUSING LENTICEL ROTS

The main fungal species responsible for the development of lenticel rots are *G. album* and *G. perennans*. It would be incorrect, however, to assume that these are the only pathogens responsible, because the following species have been isolated, but to a much smaller extent: *Cylindrocarpon mali*, *Gloeosporium fructigenum*, *Trichothecium roseum*, *Penicillium expansum*, *Fusarium* spp. and *Alternaria* spp.

DEVELOPMENT OF LENTICEL OR BITTER ROTS CAUSED BY *GLOEOSPORIUM ALBUM* AND *G. PERENNANS*

In stored apples the first signs of the disease appear in November or early December, but it has been observed occasionally on certain dessert varieties in October before picking.

Essentially the development is the same in all varieties, starting as a brown or purple discoloration of the lenticular tissues and the adjacent skin. The lesions are from 1 to 3 mm. in diameter, each having a lenticel at its focus. This stage is frequently referred to as lenticel spot. Radial expansion continues slowly until circular, flattened or concave rots (bitter rots) are formed having sharply defined margins with light brown centres surrounded by a darker marginal zone.

The ultimate size of each lesion varies according to the number present on each fruit, but finally the spore-producing structures (acervuli) break through the skin as white, wax-like bodies, normally arranged concentrically around the lenticel through which infection occurred.

Critical counts indicate that dessert varieties are more liable to attack than culinary varieties and 'king' or 'terminal' fruits are more susceptible than 'lateral' fruits.

From a superficial examination it is impossible to distinguish between rots caused by *G. album* and *G. perennans*. They can be separated readily, however, by spore examination:

G. album: Spores non-septate, hyaline, cylindrical and mostly curved, $12–27 \times 3–4{\cdot}5\ \mu$ ($21{\cdot}5 \times 3{\cdot}3\ \mu$).

G. perennans: Spores non-septate, hyaline and straight, $4{\cdot}5–21{\cdot}0 \times 2{\cdot}5–4{\cdot}5\ \mu$ ($12{\cdot}5 \times 3{\cdot}0\ \mu$).

An additional method of separation is that germinating spores of *G. perennans* produce microconidia when the germ tubes become septate, whereas those of *G. album* do not.

The rate of rotting and the rate of growth in culture of these two species is nearly identical.

THE *GLOEOSPORIUM* SPP. IN THE ORCHARD

G. album was found by Ogilvie (1935) forming small cankers on apple spurs, and the writer found it occasionally on dead pruning snags and spurs. The only certain method of detecting its presence is by incubating the suspected material in moist chambers and the examination of the white spore masses which are extruded through the bark from the acervuli lying beneath.

Many inoculations were made into wounded apple shoots, or those killed by gentle heat, but on no single occasion did the fungus establish itself.

G. perennans was first described by Zeller & Childs (1925) in the U.S.A., where it causes infection through pruning cuts, woolly aphis punctures and other bark injuries. Cankers are produced which may increase in size for several years with the formation of six or seven concentric rings of callus. Childs (1929) showed that the cankers were not invariably perennial and that new annual infections develop only when the lesions are subjected to late attack by woolly aphis or winter temperatures of -2 to $-27°$ F. Both factors are capable of damaging the limiting callus layer, so permitting a spread of infection into the surrounding healthy tissue.

The fruit-rots were found not to be serious, being usually well controlled by the adequate orchard spraying given, but Cooley & Shear (1931) reported a severe attack from one locality where late rains and spore discharge occurred before harvesting.

Brien (1932) has stated that the so-called Delicious Spot of apples in New Zealand is caused by *G. perennans*.

G. perennans was not recognized in England until 1942, when the writer recorded it causing diebacks and cankers in fruit trees in Warwickshire and lenticel rots of stored fruits (Wilkinson, 1943, 1944*a*, 1945). During the past few years, however, numerous cases have been reported of cankers and diebacks being caused by this fungus. It appears that *G. perennans* is most likely to attack the tree through wounds made in the summer, with the result that orchards of dwarf-pyramids or cordons, which receive summer pruning, are most liable to attack. Winter pruning cuts, however, appear to be less susceptible especially if made after December, and it is interesting to record that a very serious attack by this fungus, in a dwarf-pyramid orchard, was completely eliminated as the result of the substitution of winter for summer pruning.

In England, cankers formed by *G. perennans* are elliptical, slightly sunken and surrounded by a conspicuous limiting callus ring. The 'perennial' form of the canker was not observed but can be induced by artificial damage to the callus barrier.

Sporing bodies are formed readily on the exposed cortex of cankers and diebacks, especially in late summer during warm, humid weather. Isolations from these when inoculated into wounded branches form cankers very rapidly, characterized by the peeling of the bark and the blackening of the exposed cortex.

METHOD OF INFECTION OF LENTICELS BY FUNGI CAUSING LENTICEL ROTS

Ogilvie (1935) indicated that most of the fungi causing storage rots are to be found living in the parasitic or saprophytic state in the orchard. The distribution of fungal spores in orchards was investigated by Horne & Horne (1920) and Carter (1935), while Kidd & Beaumont (1924) demonstrated that stored apples have spores scattered over their surfaces but aggregated more closely in the lenticels. They indicated that 10% of the total spores on an apple may be localized in these structures.

For lenticel rots to be formed it is essential, first, for the spores to germinate; and secondly to be capable of penetrating the lenticular tissues into the apple flesh beneath. As to the exact method of penetration two opposed views are held: (1) direct penetration through the undamaged lenticels by the germinating spores; and (2) fungal penetration preceded by a physiological breakdown of the lenticular tissues. To obtain further information on this point the following experiment was carried out when microscopic examination had shown that the affected tissue beneath many of the small lenticel spots failed to reveal fungal growth.

1020 isolations were made from the affected tissue of lenticel spots occurring on Allington Pippin, the isolations being divided into the following two categories: (*a*) lesions less than 3 mm. in diameter; (*b*) lesions of 3–5 mm. in diameter. Subcultures for identification purposes were made from those isolates producing mycelial growth and, after 10 days, those remaining sterile were counted. The results are presented in Table 2.

These results suggest that in lenticel spot of Allington Pippin a high proportion of the smaller lesions are devoid of fungal growth and have been produced by agencies other than fungal. In contrast, all the lesions measuring more than 3 mm. in diameter gave mycelial growth, the main pathogen being *G. album*.

It is suggested, therefore, that the initial stage of many of the lenticel spots of Allington Pippin is non-parasitic and that spores, germinating in the lenticel craters, rapidly penetrate through the weakened lenticular tissues to form the typical circular lenticel rots.

TABLE 2. *Fungi isolated from two stages of lenticel spot of Allington Pippin*

	Lesions up to 3 mm. in diameter		Lesions of 3–5 mm. in diameter	
	Frequency	%	Frequency	%
Sterile	279	37·3	0	0
Gloeosporium album	383	51·2	214	78·7
Rhizopus nigricans	37	4·9	4	1·5
Cylindrocarpon mali	21	2·8	39	14·3
Fusarium spp.	9	1·2	—	—
Alternaria spp.	6	0·8	3	1·0
Penicillium expansum	5	0·7	—	—
Trichothecium roseum	5	0·7	8	3·0
Gloeosporium perennans	3	0·4	4	1·5

DISCUSSION

The storage-rot data given in Table 1 clearly indicate that the *Gloeosporium* spp. were the most important rot-producing organisms in apples stored at Long Ashton from 1937 to 1939, and these findings have been further substantiated by the heavy losses suffered by fruit-growers in Kent during the past 3 or 4 years. The examination of many rotted samples of fruit shows clearly that *G. perennans* and *G. album* are the chief causal organisms.

It is difficult to explain this apparent increase in the prevalence of lenticel rotting in present-day fruit stores but it may be due to one of, or a combination of all, the following factors:

(*a*) An unexplained increase in the orchard distribution and prevalence of the two *Gloeosporium* spp.

(*b*) The rapid increase in the bulk of apples stored.

(*c*) The longer storage periods now possible by the use of refrigerated gas-storage chambers.

(*d*) The tendency for modern growers to be more liberal with their nitrogenous applications.

During 1937–9 *G. album* was the principal cause of lenticel rotting, *G. perennans* being isolated only occasionally. To-day, however, a complete reversal has occurred, so that *G. perennans* is the dominant species. This may be related to the marked increase in the development of the branch lesions found in apple orchards caused by this fungus.

The problems to be faced in the future are many, but in the writer's opinion those that require immediate attention are: first, a more detailed study of the orchard ecology of the *Gloeosporium* spp. and the mode of transference of spores from branch lesions to the apple; and, secondly, a detailed study of the factors which may predispose stored fruits to attack by lenticel-rot-producing organisms. Slight evidence has been presented which suggests that a physiological breakdown of the lenticels occurs before fungal rotting develops. If this is so, certain orchard factors may play their part in predisposing stored fruits to this weakness, and such influences as variety, rootstock, manurial treatment, soil management, storage treatment and weather conditions will have to be investigated in detail before a solution to the present problem is forthcoming.

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THE RABBIT DISEASE: MYXOMATOSIS

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(With 1 Text-figure)

Introduction

Since the successful establishment of myxomatosis in the wild rabbit population of Australia in 1950, the disease has seldom been out of the news for long. Besides being an agent of biological control of a serious economic pest, an epizootic of myxomatosis offers an unusual opportunity for studying the spread of a virus disease through a susceptible population. It has frequently been suggested that the virus should be introduced into Britain, but the conditions here are very different from those in Australia; it was not known whether suitable vectors occurred here, nor whether the disease would provide a permanent form of control, and it was not desired to endanger stocks of domestic rabbits without this certainty. The symptoms of the disease are also very distressing, being characterized by an intense swelling and inflammation of the eyelids which spreads towards the forehead and ears and may also affect the genital region.

Interest was revived when it was learned in early 1953 that myxomatosis had been introduced into France the year before and had spread to many parts of the country. Australian experience suggested that the virus could 'jump' considerable distances and it seemed inevitable that it would cross from France to England. Our first outbreak occurred near Edenbridge, Kent, in the autumn of 1953 and an Advisory Committee was appointed by the Minister of Agriculture and Fisheries and the Secretary of State for Scotland and, to give the Committee time to consider the problem objectively, a determined attempt was made to prevent the spread of infection by destroying the rabbits in the Edenbridge area. Similar action was taken with the next four outbreaks but, when others were confirmed at widely separated places, the Advisory Committee came to the conclusion that the action taken to control the initial outbreaks could no longer be expected to serve any beneficial purpose. Accordingly it was discontinued.

NATURAL OUTBREAKS

Myxomatosis was first reported in 1898 from Montevideo, Uruguay, by Sanarelli, whose stock of laboratory rabbits (*Oryctolagus cuniculus*) was almost wiped out by it. Being unable to isolate a bacterium or protozoan parasite he attributed the disease to a filterable virus and named it infectious myxomatosis. Outbreaks of myxomatosis subsequently occurred among domestic rabbits in Argentina, Brazil and southern California (Kessel, Prouty & Meyer, 1931; Kessel, Fisk and Prouty, 1934), and many rabbitries were completely depopulated. Virologists have been much interested in myxomatosis and, in laboratory experiments, the mortality rate has consistently been 99·5 % or higher. The origin of the disease was unknown until 1942, when Aragão found that many of the tapetis, or native wild rabbits of Brazil (*Sylvilagus brasiliensis*), showed acquired immunity to the infection; non-immune tapetis reacted to intradermal inoculation of the virus by developing a mild form of myxomatosis with virtually no generalization and no mortality. Mosquitoes (*Aëdes* spp.) were found to transmit the virus both to other tapetis and to laboratory rabbits.

It has been suggested by Fenner (1952) that just as *Sylvilagus* is the New World equivalent of *Oryctolagus*, so is the myxoma virus the New World representative of the pox viruses; a hypothesis which is supported by the similar pathogenesis of myxomatosis and the pox diseases, and the similarity between the elementary bodies of myxoma and vaccinia viruses as shown by the electron microscope (Farrant & Fenner, 1953). In *Sylvilagus* through long association myxomatosis is a mild disease, which indicates a stabilized host-parasite relationship; but when the disease is transmitted from a *Sylvilagus* to an *Oryctolagus* the latter has no defences against it and, with rare exceptions, becomes fatally ill. The disease most closely related to myxoma is the fibroma of Shope (1932*a*, *b*), found in cottontail rabbits (the species used is not stated, but it was probably *Sylvilagus floridanus*) in eastern U.S.A. This disease resembles myxomatosis of the tapetis in causing the development of a single large tumour in infected animals, and is transmissible to domestic rabbits by implantation or inoculation. Although related immunologically to myxoma the fibroma virus is benign in domestic rabbits, and Shope (1932*b*) found that inoculation with fibroma protects them against the lethal effects of myxomatosis.

TRANSMISSION

Aragão (1920) discovered that the cat flea (*Ctenocephalides felis*) could transmit the infection, and it was later found that the Australian stickfast flea (*Echidnophaga myrmecobii*) was also a vector (Bull & Mules, 1944). Mykytowycz (in Ratcliffe, Myers, Fennessy & Calaby, 1952) showed by laboratory experiments that the rabbit louse (*Haemodipsus ventricosus*) and a mite (*Cheletiella parasitovorax*) could act as vectors. Several previous investigators (Torres, 1936; Aragão, 1942; Bull & Mules, 1944) had shown that mosquitoes could convey myxomatosis, and a laboratory study by Fenner, Day & Woodroofe (1952) proved that transmission was mechanical, since infected blood appeared to play no part; mosquitoes that had contaminated their probosces with virus from a skin lesion of an infected rabbit could transfer the disease up to 25 days afterwards, while pins stuck into the same skin lesion transferred the infection at intervals varying from a few minutes to 12 days.

SPECIFICITY

It was important that before myxomatosis was used for the biological control of rabbits there should be proof that the disease did not seriously affect any other species. Martin (1936) and Bull & Dickinson (1937) investigated this matter extensively and were satisfied that all other common domestic animals, rats, mice, marsupials, and even the European hare (*Lepus europaeus*) were insusceptible. Earlier workers (e.g. Rivers, 1926–7; White, 1929; Hyde & Gardner, 1933) had shown various mammals and birds, including the guinea-pig and the American hares *Lepus californicus* and *L. americanus* to be refractory. Cases of human encephalitis in areas affected by myxomatosis gave rise to some concern in Australia in 1952, but the association was proved to be coincidental; the two viruses are quite distinct and their only common feature is that they are both carried by blood-sucking mosquitoes. The Australian and French epizootics may be regarded as large-scale field experiments and it is notable that, other than the European rabbit, the only animals known to have been affected have been three hares in France (Jacotot, Vallée & Virat, 1953; Magallon, Bazin & Bazin, 1953).

EARLY ATTEMPTS AT BIOLOGICAL CONTROL

Although there are many examples of biological control of plant and insect pests, biological control of vertebrate pests has met with limited success. Aragão (1927) first suggested that myxomatosis might be used to control the rabbit (*Oryctolagus cuniculus*) in Australia, and the New South Wales Department of Agriculture obtained virus from him in 1926. Laboratory studies carried out by White (1929) showed that the infection could be transmitted by contact, that the excreta of a diseased rabbit were not infectious and that an artificial burrow recently vacated by diseased rabbits was not usually infectious. There were no other trials in Australia for some years, but experiments were begun at Cambridge (Martin, 1936) to determine the facility with which the disease, while maintaining a high degree of virulence, would spread through a rabbit population, kept under almost natural conditions. The results of these tests, in which the disease was spread mostly by contact between infected and healthy rabbits, were most encouraging, and were followed by extensive colony and field experiments in Australia from 1936 to 1943 (Bull & Mules, 1944). Favourable results were obtained when the rabbits were infested with the native stickfast flea and when predators were few. The most important trials, however, were done in semi-arid pastoral areas, partly because rabbit control by normal methods is uneconomic in such regions and also because the health authorities maintained a cautious attitude towards the host-specificity of the virus. These trials met with only limited success, owing to the failure of the disease to spread from one warren to another, partly due to foxes killing the slowly moving infected rabbits. Meanwhile, in Europe, three attempts were made in 1936–8 to introduce myxomatosis into a dense rabbit population on the island of Skokholm, off the coast of Pembrokeshire (Lockley 1940). The disease spread to some little extent but had no controlling effect on the rabbits and soon died out. At the same time, myxomatosis was introduced on to a Danish island in the Kattegat and an estate in Sweden, with only partial success (Jensen, 1939; Hvass & Jensen, 1939).

THE AUSTRALIAN EPIZOOTIC

A re-examination of the possibilities of myxomatosis after the war led to seven liberations of the virus being made between May and November, 1950, at five sites in the Murray Valley; four in the Albury and Corowa area and one at Gunbower 100 miles away (Ratcliffe &

Fennessy, 1951; Ratcliffe *et al.* 1952). Six of the seven liberations apparently failed to take, but the remaining one (at Balldale, Corowa, in October) flared up in December, and other reports soon came in from different parts of the Murray River. The spread of the disease was a reflexion of the movements of infected mosquitoes, and it was suggested by Ratcliffe & Fennessy that its distribution indicated an unsuspected mobility in the mosquito populations, either by long flights or passive carriage by winds. An alternative suggestion by Brereton (1953) was that the disease creeps at a fairly steady rate along the rivers.

In this first epizootic, the disease was closely associated with rivers, swamps and flood zones and was dominated by the Murray-Darling river system. It covered an area of over one million square miles and the degree of infection undoubtedly varied greatly. Along the Murray main stream adequate kills were confined to a narrow strip of a few hundred yards wide but, where tributaries provided a water network, tens of thousands of acres were almost completely cleared of rabbits. The disease was confined to the plains and with the autumn both the numbers of mosquitoes and the incidence of myxomatosis decreased. Ratcliffe *et al.* (1952) state that there is no doubt that *Culex annulirostris* and a species of *Simulium* were vectors on the Murray river, and the former was abundant in the disease area. Myxomatosis continued to be quiescent during the winter, but there was a widespread smouldering of the infection which broke out in swampy areas.

In the summer of 1951–2 high rabbit mortalities occurred over much of Victoria and New South Wales, often in areas untouched the previous year. The principal vectors were *Anopheles annulipes* and an unknown species of *Culex*, both of which spend daylight hours in rabbit warrens. State-sponsored virus inoculation campaigns may have caused greater spread of the infection (Anon. 1952; Button, 1952; Lines, 1952). Both *Anopheles annulipes* and *Culex annulirostris* breed in the lagoons of the Murray flats, but the former is a more adaptable and far ranging species. Along the Murray river the rabbit kill covered miles on either side compared with a few hundred yards of the previous summer. The weather in both the summers 1950–1 and 1951–2 was abnormal, the first providing more favourable conditions for mosquito transmission in northern New South Wales and Queensland, while the moderately dry season of the second favoured the spread of *Anopheles annulipes* in Victoria and southern New South Wales.

The season of 1952–3 was also exceptionally favourable for the breeding of mosquitoes and other insect vectors and, for the third year in succession, millions of rabbits were killed by myxomatosis in south-eastern Australia. It is estimated that four-fifths of the rabbits in this area have died, and that there has been a substantial increase in rural production as a result. For 1952–3 alone, the increase is valued at £50,000,000, of which the all-time record wool clip and increase in numbers of sheep and lambs accounts for some £34,000,000 (Reid, 1953).

The mortality rate, particularly in the first year of the epizootic, approached the 99·5% that was anticipated from laboratory studies, but the kill has not always been as high as this. Some areas showing high mortality in 1950–1 had disappointing results in 1951–2 and 1952–3, and Ratcliffe thinks that there has been a reduction in the mortality rate from 99·5 to about 92%. This means that, allowing for the persistence of recovered immune animals from one epizootic to the next, the overall kill will be 85% or less. This is the beginning of a process of host-parasite adjustment and can be due either to attenuation of the virus, an increase in the innate resistance of the rabbit, or the 'passive-resistance' of young rabbits having maternal antibodies acquired during gestation or lactation. Attenuated mutants of myxoma virus have been found in different parts of Australia, and Mykytowycz (1953) recovered from a wild rabbit at Uriarra near Canberra a strain which failed to kill any of thirteen wild rabbits infected with it by contact. The future spread of such variants is problematic and depends on their ability to multiply in the skin. Fenner, Day & Woodroofe (1952) have shown myxoma antibody to persist in rabbits for 18 months, affording protection against re-infection.

It is reasonably certain that myxomatosis will now be enzootic in wild rabbits in Australia, and in spite of changes in mortality rates it is probable that the disease will permanently reduce rabbit populations to a lower level. The present great reduction in rabbit numbers, besides increasing the stock-carrying capacity of pasture, gives graziers an unusual chance to solve their rabbit problems by eliminating myxomatosis survivors by other methods.

The French epizootic

This occurred very dramatically. In June 1952, a retired physician obtained some myxoma virus from a colleague in Switzerland, caught two rabbits from his estate in the Department of Eure et Loire, near Paris, inoculated them with virus and released them in his walled park (Delille, 1953). After a week, sick rabbits were seen and in a month most of his wild rabbits had died. He did not intend or expect the disease to spread outside his park, but whether by insect or other means it soon did so. Wild rabbits in the surrounding districts died (Jacotot & Vallée, 1953) and, within a month, domestic rabbits were also catching myxomatosis. The disease spread rapidly to other Departments, unconsciously assisted no doubt by the rabbit hunter's practice of taking rabbits from the north of France to the south to increase local stocks for subsequent shooting.

French workers were at first of the opinion that mosquitoes had not played a large part in spreading myxomatosis in their country and adduced circumstantial evidence to suggest that contaminated grass and fodder and the tyres of vehicles that had passed over myxomatous rabbits might have spread the disease. It seems likely, however, that mosquitoes or other biting insects played a key role as vectors in France as they did in Australia, and research is being done on the subject. Despite the efforts of the French authorities to stamp out the disease it had spread by the end of 1953 to every Department of France, except the three in western Brittany, and also to Belgium, Luxemburg, Germany, the Netherlands and Spain. Although myxomatosis is present virtually throughout France, by no means all the warrens have been completely depopulated. While many rabbits have been killed on the plains, warrens on higher ground have been little affected by the disease and even among adjacent fields, woods and hutches across the country, some have been infected and others not.

Myxomatosis in Britain

After the unsuccessful attempts on Skokholm in 1936–8 no further attempts were made to introduce the myxoma virus until 1952, when experiments were carried out by the North of Scotland College of Agriculture on an uninhabited island in the Hebrides. Conclusive results have not yet been obtained from this work. No official effort has ever been made to introduce myxomatosis on to the mainland of Britain for reasons outlined earlier. Since myxomatosis has spread rapidly over Europe it is evident that suitable vectors are available here, even though the species of insects responsible for the spread in Australia are not present. The first myxomatous infection of wild rabbits in England probably occurred some time in September 1953, and an outbreak at Bough Beech, near Edenbridge, Kent, was confirmed on 13 October. It is not known how the virus was brought into the country, although carriage by insects, birds or man are all possibilities. A second outbreak was confirmed a fortnight later, near Robertsbridge in East Sussex. In pursuance of the Minister's policy the centre of infection in both areas was wired with rabbit-proof netting and as far as possible all rabbits within the netting were killed. Since the beginning of November nine* further outbreaks have been confirmed, as follows:

Alciston, East Sussex	2 November 1953	Lydd, Kent	5 December 1953
Sevenoaks, Kent	20 November 1953	Faversham, Kent	4 January 1954
St Osyth, Essex	27 November 1953	Great Totham, Essex	27 January 1954
Southwold, East Suffolk	2 December 1953	Boughton Aluph, Kent	2 February 1954
Holland-on-Sea, Essex	5 December 1953		

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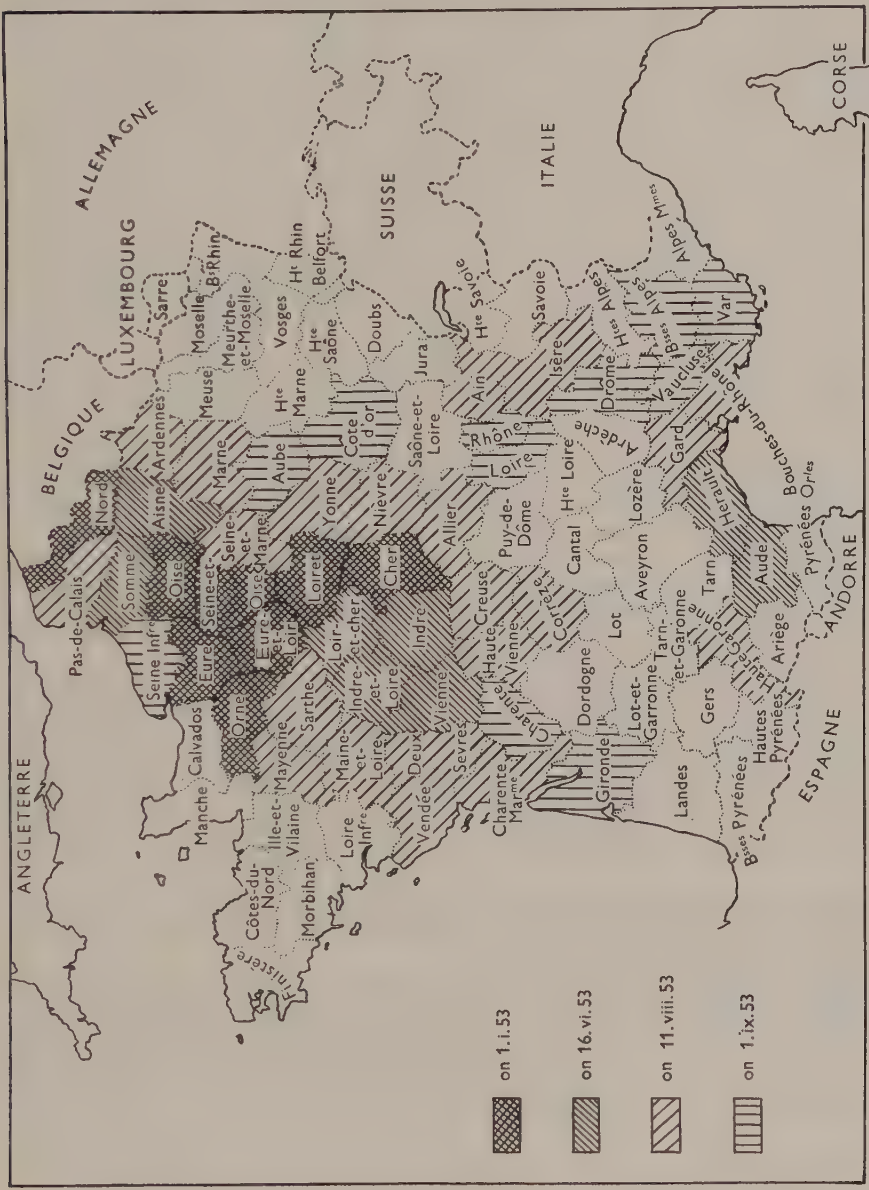
* Since the meeting a further outbreak has been confirmed at Wateringbury, Kent, on 2 March.


ANGLETERRE
BELGIQUE
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ALLEMAGNE
SUISSE
ITALIE
ESPAGNE
ANDORRE
CORSE
Pas-de-Calais
Nord
Somme
Aisne
Ardennes
Oise
Seine Infre
Seine-et-Oise
Seine
Seine-et-Marne
Marne
Meuse
Moselle
Sarre
Meurthe-et-Moselle
B. Rhin
Ht Rhin
Vosges
Belfort
Hte Marne
Hte Saône
Doubs
Jura
Aube
Yonne
Cote d'or
Saône-et-Loire
Nièvre
Loiret
Cher
Allier
Eure
Eure-et-Loir
Calvados
Manche
Orne
Sarthe
Mayenne
Ille-et-Vilaine
Côtes-du-Nord
Finistère
Morbihan
Loire Infre
Maine-et-Loire
Loir-et-Cher
Indre-et-Loire
Indre
Vienne
Deux Sevres
Vendée
Charente Marme
Charente
Haute Vienne
Creuse
Puy-de-Dôme
Loire
Rhône
Ain
Hte Savoie
Savoie
Isère
Drome
Htes Alpes
Bsses Alpes
Alpes Mmes
Var
Vaucluse
Bouches-du-Rhone
Ardèche
Hte Loire
Cantal
Corrèze
Dordogne
Gironde
Lot
Lot-et-Garonne
Tarn-et-Garonne
Aveyron
Lozère
Gard
Herault
Tarn
Aude
Pyrénées Orles
Ariège
Haute Garonne
Gers
Landes
Hautes Pyrénées
Bsses Pyrénées
on 1.i.53
on 16.vi.53
on 11.viii.53
on 1.ix.53

Fig. 1. Spread of myxomatosis in France in 1953. (Reproduced by permission of the Conseil Supérieur de la Chasse.)

It was quite impracticable, for one reason or another, to wire in the affected rabbits in most of these outbreaks, and it became clear that the destruction of rabbits by gassing and other means in these areas was unlikely to succeed in stamping out the disease in this country. It was even possible that drastic thinning of the population by slowing down the rate of infection might actually assist the disease to overwinter. It was therefore decided that no useful purpose was being served by the attempts being made to stamp out the disease. Accordingly, in all but the early centres of infection, it has been allowed to take its natural course.

Despite the spectacular reduction in rabbit numbers in France and eastern Australia, the increasing number of immune rabbits in the latter country indicates that the Australians may have by no means seen the last of their rabbit problem. Although introductions of myxomatosis into Tasmania and New Zealand have met with local successes, there have not been any epizootics, and it seems probable that they will only occur when the climatic conditions establish a favourable host-vector relationship. Because myxomatosis arrived here in the autumn, it was thought that the disease would probably be quiescent during the winter and might even die out, since sylvan mosquitoes which bite rabbits overwinter only as eggs or larvae. The disease has, however, managed to maintain itself and even to spread slowly. An epizootic in this country is quite likely to begin during late spring and early summer and there is urgent need for research into the means of transmission and rate of spread. Under the heading of transmission, it is proposed to investigate the incidence and ecology of mosquitoes, fleas and other blood-sucking arthropods associated with the rabbit in different parts of Britain. By means of a serological test known as the precipitin test, the host from which an engorged insect obtained its blood meal may be determined, and it is proposed that insects should be collected from various rabbit habitats and the sources of their meals identified. The different species of insects collected from areas where myxomatosis is present will also be tested for infectivity in the laboratory and when it is more fully known what species of arthropods feed on rabbits in the wild, their efficiency as myxomatosis vectors can also be tested. The infectivity of herbage, excreta and other means of indirect contamination will also be investigated.

By studying natural outbreaks and estimating rabbit populations in the immediate neighbourhood and by catching, marking and releasing rabbits, it is hoped to obtain information on the home range and distances travelled by rabbits. This should provide information on the spread of myxomatosis by direct contact or by ecto-parasites and it is hoped to correlate the rate of spread with the means of transmission. Particular attention will be paid to rabbits surviving in myxomatosis outbreak areas and the amount of immunity in the population at different times will be determined. In co-operation with the North of Scotland College of Agriculture, the artificially induced outbreak in the Hebrides will be further studied.

Work is already proceeding at the Ministry's Veterinary laboratories on the preparation of vaccines for the protection of tame rabbits, and it is hoped to improve their quality and the duration of the immunity conferred by them.

An epizootic in Britain may be expected to reduce rabbit numbers to a very low level, but some equilibrium between virus and rabbit is sure to be established eventually and the need for intensive control measures of every sort, in conjunction with scrub clearance and other land improvement measures, will be more than ever essential if rabbit numbers are to be permanently reduced.

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OBITUARY NOTICES

TOM GOODEY, O.B.E., D.Sc., F.R.S.

Tom Goodey died suddenly, from a heart attack, on 7 July 1953. For over 30 years he had worked in a much neglected field of applied biology, studying the free-living and plant-parasitic nematodes and the effects of the latter on their hosts. In this field he had long enjoyed a high international reputation, and he played no small part in getting nematology recognized in this country as an important branch of economic biology.

Born at Wellingborough on 28 July 1885, Goodey was educated at Northampton County School and the University of Birmingham, whence he graduated with honours in zoology and botany in 1908. After some sound early work in marine zoology he specialized in soil protozoology at both Rothamsted and Birmingham, from 1912 to 1919. He spent 1 year (1920) as plant helminthologist at Rothamsted, and then joined Leiper's staff at the London School of Tropical Medicine. Here, for 5 years, he worked mainly on nematode parasites of vertebrates but partly on free-living and plant-parasitic species. The latter, the 'eelworms' of nematology, absorbed most of his attention after 1925 when he joined the new Institute of Agricultural Parasitology at St Albans. He was the Principal Research Assistant there for 22 years and, when the Institute was disbanded on Leiper's retirement in 1947, its nematological part became the new nematology department at Rothamsted with Goodey as Head until 1952 when he retired from the headship to give his whole time to the work he best liked doing. He was revising the genus *Anguina*, re-writing his first text-book, and planning a visit to Australia to advise the government there on nematological research, when he died.

Goodey was pre-eminently a taxonomist, as his long series of finely illustrated papers shows. Conservative in systematics—a lumper rather than a splitter—he was progressive in other sides of his work, constantly devising new techniques and adopting or adapting those of others. But he was no narrow taxonomist of the 'preserved specimen' school: always he showed a keen interest in the living animal and its life history, and in the interrelationships of parasite and host. This is clear in his two text-books, *Plant Parasitic Nematodes* (1933) and *Soil and Freshwater Nematodes* (1951). He worked mainly on the plant-parasitic Tylenchs and Aphelenchs, and on free-living nematodes, but his biological flair shows best in the *Phil. Trans* (1930) paper on *Tylenchinema oscinellae* parasitic in the frit fly, where he unravelled the complex interactions of oat, insect and nematode.

Besides the text-books, upwards of 120 published papers stand as a lasting memorial to his scientific work. He had a protozoological note in the first volume of these *Annals* (1915) but, apart from six later papers, his work was published elsewhere. He joined the Association in 1920, served twice on the Council, and was President in 1934–5. Beyond scientific interests, Goodey had long been an active member of the Society of Friends; he also had a fine tenor voice and throughout adult life delighted public and private audiences with his clear interpretation of folk song, German lieder, opera, and oratorio.

As a scientist Goodey was keen-sighted, patient and industrious. His many friends will remember his great sincerity, integrity and humility. Behind all he undertook was the drive of a whole-hearted and infectious enthusiasm. Slow to anger, he could be quite terrifying when roused. Lest this sound bleak and solemn, it must be added that near the surface was an overmastering sense of fun, frequently breaking through to prick bubbles of pomposity.

He leaves a widow, a son and three daughters. His colleagues at Rothamsted and scattered throughout the world, and his many friends, sadly miss him, but are thankful for having been privileged to know him.

B. G. Peters

MISS WINIFRED BRENCHLEY, O.B.E., D.Sc., F.L.S.

With the death of Dr Winifred Brenchley on 27 October 1953, the botanical world lost a much valued personality. Born in 1883, she was brought up in London and educated at the James Allen School, Dulwich. After obtaining her diploma at Swanley Horticultural College, she went to University College, London, where, despite a serious operation which left her permanently deaf, she obtained a first-class honours degree in botany in 1905. Her doctorate followed in 1911, and she was made a Fellow of University College in 1914. Awarded the Gilchrist Scholarship for University Women in 1906, she came to work at Rothamsted, where she was appointed on the staff in the following year, and remained as Head of the Botany Department until 1948. On her retirement she received the O.B.E.

Her publications were numerous and covered a wide field. She soon became interested in the effects of various chemical elements on plant growth and in this connexion did much to develop the water-culture technique. These early investigations led to the publication in 1914 of *Plant Poisons and Stimulants*, in which she rightly forecast the possible practical importance of some of these elements in agriculture. Although micronutrient problems continued to be one of her main lines of research, she never lost touch with the more general aspects of plant nutrition. Field work always attracted her, fostered no doubt by her horticultural training and her association with Prof. Oliver at University College, and she devoted much time to a survey of weed floras on farms in different parts of England, the results appearing as *Weeds of Farm Land* in 1920. This interest in weeds was maintained throughout her career, and later she collaborated with H. C. Long in the production of *Suppression of Weeds by Fertilizers and Chemicals*, which reached its third edition in 1948. Her name will also be associated with the long-term study she made of the botanical composition of the permanent hay plots at Rothamsted, data prior to 1920 appearing in her monograph *Manuring of Grassland for Hay*.

Besides being a competent biologist, Dr Brenchley possessed great mathematical ability and she was quick to appreciate the importance of modern statistics. Methodical by nature and with unusual powers of concentration, which enabled her to accomplish an impressive amount of scientific work, she also found time to take an active part on many committees, was elected Vice-President of the Association of Applied Biologists in 1940–1 and served on their Council 1940–2. Always ready to help, her advice was instinctively sought by old and young alike and she was a tower of strength in the administrative, social and domestic affairs at Rothamsted. Her standards were high, but her criticism was invariably constructive and kindly, and she was generous in giving credit to the younger worker. In spite of her deafness, she enjoyed social occasions and had a great capacity for making new and keeping up with old friends both at home and overseas. Her interests were many and varied, entomology, gardening and philately in particular occupying her leisure hours. She loved to travel and it is sad that her years of retirement, to which she had looked forward so much, were mostly spent in a long and incapacitating illness.

Apart from her scientific work, which earned her a well-deserved reputation, she will long be remembered for her generous spirit, sound judgement and lovable character.

K. Warington

REPORT OF THE COUNCIL OF THE ASSOCIATION OF APPLIED BIOLOGISTS FOR THE YEAR 1953

The Officers and other Members of the Council of the Association were as follows:

President: Prof. W. Brown, M.A., D.Sc., F.R.S.
Vice-Presidents: Prof. J. B. Cragg, M.Sc.; R. W. Marsh, M.A.
Hon. Treasurer: H. F. Barnes, M.A., Ph.D.
Hon. Gen. Secretary: R. K. S. Wood, A.R.C.Sc., Ph.D.
Hon. Programme Secretary: L. Broadbent, B.Sc., Ph.D.
Hon. Editor of the *Annals of Applied Biology:* R. W. Marsh, M.A.
Hon. Asst. Editor: I. Thomas, M.Sc., Ph.D.
Other Members: P. W. Brian, M.A., Sc.D.: C. H. Cadman, B.Sc., Ph.D.; Prof. J. B. Cragg, M.Sc.; D. W. Fenwick, D.Sc.; R. M. Greenslade, B.Sc., Ph.D.; I. Isaac, B.Sc., Ph.D.; W. F. Jepson, O.B.E., A.R.C.Sc., Ph.D.; Miss M. A. Keay, M.A., Ph.D.; A. H. McIntosh, B.Sc., Ph.D.; B. D. Moreton, B.Sc.; A. F. Posnette, M.A., Ph.D.; I. W. Prentice, M.Sc., Ph.D.

During 1953 forty-nine new Members were elected, two old Members rejoined and the following four resignations were accepted: G. O. Evans, H. L. Fisher, J. C. McGowan and F. J. Newhook. On 31 December 1953 membership stood at 670 but the following nineteen Members resigned from the end of the year: J. R. Audy, Miss A. B. Beakbane, A. Beaumont, Miss M. Bennett, Miss C. A. Blair, G. A. J. Carter, Mrs K. Crawford, H. A. Dade, L. D. Galloway, W. H. Goddard, F. G. Gregory, Miss J. R. Groves, F. Y. Henderson, G. C. Johnson, Harbans Lal, G. B. Lush, A. P. D. McClean, C. R. Metcalfe and P. J. Rennie, and the following seven Members are presumed to have resigned: S. W. Bailey, A. D. Burman, R. H. E. Kips, N. Landau, T. D. Mukerjea, Miss J. L. Smith and H. E. Street.

The Council regret to announce the deaths of Dr T. Goodey, Miss W. E. Brenchley and Dr W. A. R. Dillon Weston. Dr Dillon Weston was for thirty years the Advisory Mycologist for the eastern counties and will be specially remembered for his work on mercurial seed dressings. Obituary notices of Miss Brenchley and Dr Goodey appear on pp. 367–8, Vol. **41**, of the *Annals of Applied Biology*.

The Council noted with pleasure the conferment of the honour of O.B.E. on Dr T. Whitehead, Dr F. J. Nutman and Dr T. Small during 1953.

Seven senior Members of long-standing availed themselves of the opportunity under Law V of becoming Life Members during the year.

Four paper-reading meetings were held in London with an average attendance of ninety Members and thirty-eight visitors. A provincial meeting was held in Bristol on 13 November, some forty Members and twenty visitors attending. Details of these programmes are as follows:

27 *February*. Symposium on the assessment of plant pests and diseases. Chairmen: R. W. MARSH and I. THOMAS. E. C. LARGE: Some recent developments in fungus disease survey work in England and Wales. H. E. CROXALL: The assessment of losses caused by apple diseases with special reference to apple scab. R. HULL: Assessment of disease incidence in the sugar-beet crop. A. H. STRICKLAND: Field technique in pest assessment. F. G. W. JONES: Assessment of economic loss from pests defoliating sugar beet. D. W. WRIGHT: The assessment of damage caused to some brassica crops by the cabbage root fly.

17 *April*. Annual General Meeting. Presidential Address, R. W. MARSH: The past and the future of *The Annals of Applied Biology*.

2 *October*. Symposium on organo-phosphorus insecticides. Chairmen: V. B. WIGGLESWORTH and J. R. NICHOLLS. K. A. LORD and C. POTTER: Insecticidal and anti-esterase activity of organo-phosphorus compounds. J. M. BARNES: Toxic action of organo-phosphorus insecticides in mammals. G. S. HARTLEY: The behaviour of organo-phosphorus systemic insecticides in the living plant. P. R. CARTER: Some hydrolytic aspects of organo-phosphorus compounds. J. F. NEWMAN: Bio-assay of organo-phosphorus.

11 *December*. M. TVEIT: Control of seed-borne diseases of cereals with *Chaetomium* spp. D. J. GRIFFITHS: Problems of breeding for disease resistance in oats. F. T. LAST: The spore content of the air in cereal crops. J. H. WILLIAMS: The effect of molybdenum on reclaimed Welsh upland pastures. J. R. W. JENKINS: Seed dressing as a means of controlling insect pests. R. GAIR: Frit fly assessment and control. B. JOHNSON: The influence of flight on host selection and reproduction in aphids.

13 *November*. The Provincial Meeting was held in Bristol on 13 November in the Physics Lecture Theatre, Royal Fort, University of Bristol. The following papers were read: I. ISAAC: Studies in the spread and control of diseases caused by *Verticillium* spp. L. E. W. STONE: Infestation of delphinium roots by the eelworm *Pratylenchus pratensis*. H. G. MORGAN: *Napomyza lateralis:* a new pest of calendulas. A. IBBOTSON: Strawberry weevil (*Otiorrhynchus clavipes* var. *fuscipes*) in the Cheddar area. D. L. ABBOTT: Recent applications of growth substances in fruit growing. J. T. MARTIN, R. W. MARSH and N. G. MORGAN: Spray application problems. D. A. GOVIER: Observations on chrysanthemum virus diseases. Luncheon was served in the Refectory and Tea in the Senior Common Room. The Council wish to record their thanks to the University authorities for providing facilities for the meeting and to Mr R. W. Marsh and Mr N. G. Morgan who were responsible for the arrangements.

At the kind invitation of the Directors of F. W. Berk and Co. Ltd., the Spring Meeting of the Association was held at the Tilgate Research Station near Crawley, Sussex on 13 May. The meeting which was attended by some fifty Members was organized by Dr J. R. Booer, Director of Research for the Company. Members travelled down by special coach and were met on arrival by Dr Booer and other representatives of the firm. They were entertained to luncheon at the Tilgate Forest Hotel and then spent the afternoon seeing the work in progress at the station. This was primarily concerned with the investigation of the biological properties of the Company's products which include compounds of mercury and copper, sulphur preparations, formulated insecticides based on D.D.T., Lindane and derris, and fertilizers. Members were entertained to tea later in the afternoon; the President, Prof. Brown, on behalf of Council and the Association, warmly thanked the Company for their hospitality and Dr Booer and his colleagues for organizing so enjoyable and instructive a meeting.

The Summer Meeting, arranged to take place in Aberystwyth on 13–17 July, was cancelled at the last moment owing to lack of support; only sixteen Members and guests stated they would attend. The majority of the papers to have been given at this meeting were read at later meetings of the Association.

During the year, the Sub-Committee of Council dealing with arrangements for the Jubilee Conference of the Association to be held on 13–17 September 1954 met a number of times, and by the end of the year had completed the main arrangements for this series of meetings. Leading workers in applied biology from the Commonwealth, Western Europe and North America will review the progress of the past fifty years; plans have also been made for a Conversazione, a Reception, a Dinner, and for visits to Research Stations in south-east England and the Midlands. Dr Jepson was appointed Assistant General Secretary to help with arrangements for the Conference. Details of the Jubilee Fund as it stood on 31 December 1953 will be found in the Treasurer's Report for 1953.

The Association was represented on the Biological Council, the Parliamentary and Scientific Committee, the National Committee for Biology and the British Standards Institution Technical Committee PCC/1, Nomenclature for Pest Control Products.

The Council wishes to thank Miss B. M. Stokes for continuing the distribution of copies of *Common Names of Insect and Other Pests.*

In conclusion, Council wish to thank once again the Governing Body of Imperial College for their kindness in providing lecture theatres for meetings and in permitting use of their catering facilities.

REPORT OF THE HONORARY TREASURER FOR THE YEAR ENDING 31 DECEMBER 1953

Income from the sale of the current volume of the *Annals of Applied Biology* amounted to £2356, a decrease of £6 over the sale of the *Annals* in 1952. The income from the sale of back volumes and parts was £278, a decrease of £28, while the sale of reprints and advertisements showed an increase of £123. The cost of production was £3716, an increase of £427. The net cost of the *Annals* was £798 as against £447 in 1952.

The amount received during the year for subscriptions including arrears was £1037, an increase of £286. This is the result of increasing the membership subscription rate by 10*s.* and abolishing the entrance fee.

Income for the year showed an excess of £166 over expenditure as against £201 in the previous year. In the main this is accounted for by the increased net cost of the *Annals* being largely offset by the increase in subscriptions.

The Association is once again indebted to the Royal Society for a grant of £300 from the Government grant-in-aid. It also acknowledges with thanks a special grant of £45 paid through the Crown Agents for the Colonies.

The Jubilee Fund received seven donations and amounted to £146 at the end of the year, but many more donations are needed to ensure an adequate covering of the expenses of the forthcoming Jubilee meeting.

The net assets of the Association at the end of the year amounted to £3310, an increase of £460. All available surplus funds are invested in National Savings Certificates and 2½% Defence Bonds and on deposit at the Post Office. The estimated value of the stock of the *Annals of Applied Biology* is £214. 7*s.* 0*d.*

The position of the Association is at present satisfactory although, in view of the increasing cost of production of the *Annals*, there still remains the need for extreme watchfulness on the finances of the Association.

REPORT OF THE HONORARY EDITOR FOR 1953

To reduce the delay in publication of papers, vol. **40** was expanded to 793 pages and 8 plates as compared with 626 pages and 23 plates for vol. **39**. At the end of 1953 the period between receipt of papers and their publication was approximately 11 months.

Including contributions published in *Proceedings*, vol. **40** contained 67 papers, the subjects being roughly classified as follows: general applied botany, 7; mycology and plant diseases, 24; viruses and virus diseases, 8; entomology and insect pests, 15; insecticides and fungicides, 10; nematology, 3. With the exception of two papers in *Proceedings* no papers by non-members were accepted in 1953.

The past and the future of the *Annals of Applied Biology* formed the subject of the Presidential Address, published in the September number.

The members of the Editorial Committee remained unchanged in 1953: their services and those of the Assistant Editor are gratefully acknowledged.

ASSOCIATION OF APPLIED BIOLOGISTS, PLANT PESTS AND DISEASES COMMITTEE, REPORT FOR 1953

One meeting has been held, on 1 October 1953. H. Jacob, J. E. Crosse and A. H. McIntosh were appointed by Council to fill vacancies caused by the retirement of members whose term of office had expired. J. B. Goodey and E. Dunn were re-elected Chairman and Secretary respectively.

Copies of the list of different varieties, drawn up by the Sub-Committee appointed to collect information relating to the susceptibility of fruit varieties to damage by spray materials, have been circulated to a number of provincial fruit specialists for comment and criticism.

A Memorandum on proposed alterations to the Constitution of the P. P. and D. Committee was submitted to Council for consideration. Following further discussion, Council and members of the Committee agreed that the P. P. and D. Committee be discontinued as a Standing Committee and that its place be taken by Temporary Committees formed as the need arises, to consider and report on special problems formulated by Council or members of the Association.

Ann. appl. Biol. **41** (2), 374–378.

REVIEWS

The Anatomy of the Migratory Locust. By F. O. ALBRECHT. Pp. 118. London: The Athlone Press. 1953. 30*s.*

Mr Albrecht's book contains a detailed account of the external and internal anatomy of the locust, and should prove an extremely useful work of reference to entomologists using the locust for experimental biology or physiological studies. An appendix on instructions for dissection is included, thus making the book an excellent aid to teaching if the locust is used as an insect type.

The volume is well produced, with large clear print and good, well-drawn diagrams.

A. STRINGER

Insecticides and Colonial Agricultural Development. Edited by T. WALLACE and J. T. MARTIN. Pp. 169. London: Butterworth's Scientific Publications. 1954. 30*s.*

The Colston Research Society, the scientists, the recorders, the editors and the printers, all who had a hand, however small, in the production of this volume are deserving of the gratitude of everyone interested in colonial development. This symposium, although it is only the sixth, held by the Colston Society, happens to be the second devoted to colonial matters; Colonial Administration was the subject of volume 3 of the Colston papers published in 1950.

The Society—originally the University College Colston Society—was formed in 1899 by a group of public-spirited Bristol citizens and was named after Edward Colston, a well-known seventeenth-century philanthropist and educationalist. At first it collected annually a sum of money for fostering research, but in recent years the money has been devoted to the promotion of an annual symposium.

Over eighty leading scientists, mainly entomologists and plant-protection chemists, took part in this symposium. They were drawn from the colonies, from research stations and universities in Great Britain, and from the scientific and technical staffs of leading commercial firms concerned with the supply of insecticides and equipment to the colonial territories. The names of a number of these leading scientists are household words in applied biology, and all the contributors show that enthusiasm and attention to detail which is the hall mark of the research worker whether in the field or in the laboratory.

Dr W. J. Hall, from a vast fund of experience, contributes a paper which forms an admirable review of the subject of crop protection in the colonies. It is full of extremely interesting facts and figures and it deserves to be very widely read—not only by entomologists. From this paper we learn, for instance, that there are thirty-eight colonies covering about 2¼ million square miles and supporting a population of 70 million people; that exports from the colonies include £49 million worth of cocoa, £32 million worth of sugar, £12 million worth of palm oil and £40 million worth of sisal; that the northern region of Nigeria produces annually a crop of 138,000 tons of maize valued at £2,620,000 and 1,729,000 tons of sorghum valued at £26,800,000. Dr Hall also gives very considerable information about crop losses in the colonies, where the toll exacted by insects is considerably larger than that generally experienced in Great Britain. Other instances are given of very large sums of money that have already been saved by the work of entomologists: the introduction into the Fijian island of Vita Levu of a Tachinid fly parasite of the coconut moth which saved a potential loss of annual income of some £300,000 (at present prices £2½ million); and the control of the cotton jassid in Anglo-Egyptian Sudan, which resulted in an estimated increase in yield valued at between £4 and £5 million.

Dr Hall concludes that the numbers of scientists and technicians engaged in combating pests and diseases are quite inadequate to deal with such colossal problems. He emphasizes that there is a need for fundamental research work as well as 'development' work—the correct identification of the pests involved, the study of their life history and habits, development of suitable machinery and the introduction of new insecticides and the study of the problems involved thereby.

C. B. Symes, in a paper entitled 'Some Observations on Colonial Insecticide Research', also outlines the magnitude of the losses caused by the pests in relation to the paucity of our efforts to redress the balance. He outlines in some detail both the crop pests that have been tackled in the field and the research that has led up to some of the useful control measures now being used. But Mr Symes, too, ends up on the same note as Dr Hall in emphasizing the need for more research work and more scientists. To make a good start, he says, we need 'eight or nine teams each of two entomologists, an insect physiologist, a chemist, a physicist, a botanist or plant physiologist and an engineer'.

The remainder of the papers are of a more specific nature, the subjects dealt with including cotton pests, locusts, spraying machinery and pests of livestock. Prof. Munro emphasizes the losses caused by insects of stored products in relation to the ever-increasing population of the world. He points out that the periodic famines that have occurred in the past may occur again on a bigger scale, and though we know the causes of these famines no serious attempt has been made to anticipate famines following bad seasons.

Although the symposium was concerned largely with the role of insecticides in colonial development, a warning note is sounded by Mr Fennah who rightly emphasizes that opportunities for major advances present themselves only when the most important limiting factors can be removed. In dealing with the seriousness of insect losses in any part of the world all entomologists would do well to remember this most important principle. In the West Indies nowadays, for instance, Mr Fennah says that the limiting factor is 'retarded or unbalanced metabolism of the product, whether plant or animal'. Current agronomical practice does not adequately overcome the difficulties imposed by the physical characteristics of the environment and this limitation is of overwhelming importance in the Caribbean territories compared with the depredations by insects.

A warning note of a different nature is sounded by Mr Way in a paper in which he shows the dangers of a short-sighted policy of applying an insecticide to plants maintaining a nicely balanced community of insects.

The final paper is by Dr Holmes, and this, too, should be widely read. With the development of keen competition in the international field it is only right that the research worker should know and appreciate the manufacturer's problems and have some idea of what it costs to discover, develop and market a new insecticide.

Dr Holmes's last paragraph deals with the good relations that now exist between 'official research workers' and those in industry. A glance at the list of members present at the symposium and the accounts of the discussions that follow the papers is evidence of this, and indeed it is difficult nowadays to think why relations should ever have been strained or difficult.

I. THOMAS

Diseases of British Grasses and Herbage Legumes. By KATHLEEN SAMPSON and J. H. WESTERN. Second edition. Pp. xiii+118. Cambridge University Press. 1954. 15*s.* net.

It is difficult to realize that almost fifteen years have elapsed since the first edition of this book appeared. During that time, however, 'Sampson and Western' has become a *vade-*

mecum for the study of diseases of grasses and herbage legumes, a subject of paramount importance to British agriculture. In the second edition the work has been brought well up to date, the authors having made good use of information from many sources to supplement their own experience and published accounts of diseases.

Concerning nomenclature, those of us who are not yet familiar with the genera *Anisantha* and *Zerna* will be grateful that the old name *Bromus* is also provided. *Calonectria graminicola* should, however, now be replaced by *C. nivalis*.

It is to be hoped that the appearance of this book will stimulate further work on the subject, particularly by surveys of pasture plant diseases. For instance, in the 1940 edition it was stated authoritatively that *Colletotrichum trifolii*, the cause of a serious disease of lucerne in America was unknown here. Now the authors report that it was found in Kent in 1950 'and on a few occasions since'. Surveys of field crops and of seed supplies might indicate how this disease was introduced and how it could be controlled or even yet eliminated.

Careful editing and a twenty-page bibliography make this book a much more fertile source of information than its ninety pages of actual text would lead one to expect. The authors and publishers are to be congratulated on their work. MARY NOBLE

Principles of Biochemistry: A Biological Approach. M. V. TRACEY, M.A. Pp. 194. London: Sir Isaac Pitman and Sons Ltd. 1954. 20*s*.

It is an interesting, and perhaps a little disquieting, reflexion that the subtitle of this book—'A Biological Approach'—should be considered necessary. What, one asks, could be expected of biochemistry but that it should be approached biologically? The fact is, of course, that not only the approach to, but also the content of, biochemistry has become increasingly chemical and decreasingly biological. And this must be so, because to questions framed in biological language, the answers are so frequently provided in chemical terms.

Mr Tracey's book is fairly difficult reading. It is much more an essay, a development of a point of view, than a text-book. A primer it certainly is not, for it presupposes in the reader an already ample equipment of biochemistry, and not only the text, but also the figures and tabular insets which fulfil the function of illustrations, have to be worked at to be grasped. Nevertheless, the striving after an understanding of every aspect of the chemistry of organisms in terms of function, and of functions in terms of chemistry, is a notable feature of the book, and one which makes its reading well worthwhile. Above all, in the reviewer's opinion, the effect on the reader must be to get a feeling of how bodies work, and, perhaps still more important, how they do *not* work; and that, it seems, is what the author has set out to do in writing it. Perhaps the construction is a little too logical, the treatment too dry, to carry much in the way of feeling; but some of the chapters, especially those in the second part dealing with such themes as the environment and competition between organisms, are admirable both in prose style and in subject-matter.

To the biologist, therefore, this book will not only provide, with all the advantages of brevity, an integrated view of biochemistry, but will also reveal the form and language in which the answers to his questions are increasingly being framed. E. C. BATE-SMITH

Information Theory in Biology. Edited by H. QUESTLER. Pp. 273. University of Illinois Press. 1953. $4.00.

The organizers of this collection of essays have attempted something both novel and useful but, for the most part, the attempt has not been successful. In places the work has a surrealist air, and one wonders whether it is a hoax got up by a professional humorist to make fun of 'science'. But then one moves on to a more sensible essay and realizes that it is all meant seriously.

There are four sections. The first is nearly confined to straightforward information theory and to a brief statement of the relation between 'information' and entropy. It is weakened at the end by a discussion of enzyme specificity which is so oversimplified as to disregard the existence of enzymes that act on a range of substrates but at diverse rates. The second section is on protein structure and contains most of the gems. Thus we are told that 'It is an experimental fact that the biological action of different proteins of the same constitution is the same', which appears to be a tautology. Again, thirteen pages are devoted to an unsuccessful search for any tendency that amino-acids may show to occur as neighbours, but the authors admit that their method, when applied to sentences in English, does not reveal the well-known connexions between different letters of the alphabet. Throughout this section the assumption appears to be made that a protein with a particular enzymic or other activity is a unique structure, and no attention is paid to the growing evidence that there is some elasticity in their composition.

The third section deals with antigenicity and genetics and contains some interesting material that has only recently been published. This also is marred by overstatements of the amount of specificity that has actually been observed.

Finally 'Biosystems' are considered with special reference to the control of the blood-sugar level and of twinning. The section goes on to discuss the total entropy, or information, content of bacteria and people. In places this takes one back to the argument about 'energy of organization' that went on 30 years ago, but conclusions are cautious and the authors of these essays seem to realize the irrelevance of the issues with which the authors of the second section were concerned.

N. W. PIRIE

Die Larvenformen der Dipteren. By W. HENNIG. Vol. III. Pp. 616 with 338 text-figures and 21 plates. Berlin: Akademie-Verlag. 1952. 15.60 U.S. $.

Authors who attempt major works which are to be published in two or more parts are readily divided into two categories—those who enthusiastically embark on Vol. I and then realize that the task is too formidable to be completed and those who complete their self-appointed task and to whom the scientific world ever afterwards owes a debt of gratitude. Dr Willi Hennig belongs to the latter category.

Vol. III of this work is much bigger, and to the economic entomologist, much more interesting than Vols. I and II. It deals with the whole of the Brachycera which is divided into the Tabanomorpha and Muscomorpha; the former is then subdivided into the Tabaniformia and Asiliformia and the latter into the Empidiformia and Musciformia (Cyclorrhapha). This subdivision and the classification into families is not altogether in line with that of Colyer and Hammond, but the family names in the two books are closely related enough to be easily recognizable to all students of Diptera. Some idea of the ground covered by this volume may be appreciated from the fact that the Chloropidae for instance occupy less than eleven pages, and that very important family the Muscidae (including the Anthomyiidae) only about forty pages.

The very nature of this work and its magnitude has called for keen discriminatory powers, and it has been evident in this and the previous volumes that the author has mastered his subject completely. The drawings are clear and adequate—there are 338 text-figures and 21 plates—the references to the literature are very full and up to date, the print is clear and is on reasonably good paper. This and the previous volumes are, however, bound only in very flimsy covers; and, expensive though they are, all students of Diptera will find them indispensable.

I. THOMAS

Manual of the North American Smut Fungi. By GEORGE WILLIAM FISCHER. Pp. xii + 343. New York: The Ronald Press Company. 1953. $8.75.

Those who have used Prof. Fischer's book on the Bibliography of the Smut Fungi will know how thoroughly he deals with his subject. This second book is a taxonomic treatment of the smut fungi found in the North American continent.

The manual covers 22 genera, 276 species, including several varieties on species of 242 host genera. Rather more than a quarter of the smut species are known in this country. There have been several consolidated species and one new description (*Ustilago sonoriana* Zundel on *Muhlenbergia dumosa*). The concept of species is based primarily on morphology. Where, however, two or more species of similar morphology are known on hosts of different families, they are maintained as distinct.

In writing the book the author's aim was 'to enable persons with some botanical and mycological background to identify with reasonable certainty any of the species of smut fungi known to occur in North America'. For this purpose numerous keys are provided, all adopting the dichotomous arrangement. The host index (with the genera in alphabetical order) and key to the smut species covers twenty-eight pages. A shorter key to the smut genera based on spore and sorus appearance and morphology is also given. A key to smut species is provided under each genus, and genera and species are in alphabetical order throughout. In addition, at the end of the book, a complete host and smut species index is given. The author certainly has done everything possible for easy and quick reference.

The synonyms of each species are given, with a description of sori and spores, followed by notes on the host range and distribution. No reference is made, however, to the importance of the species. Most of the descriptions of the species are based on original study of type or authentic material and the abundance of microphotographs, which with very few exceptions are excellent and true to scale, are of the same material. Spores of nearly all the species are illustrated, and in some cases host infection also.

The book is very well produced on art paper and is an excellent contribution to our knowledge of the distribution of the smut fungi. C. C. V. BATTS

Sex-determination. By F. A. E. CREW. 3rd ed. Pp. 54. London: Methuen's Monographs on Biological Subjects. 6*s.* 6*d.* net.

Compressed into 54 pages of text a surprising amount of useful information is found and a bibliography, glossary and indices add to the value of the little book. The problems of sex differentiation are deliberately excluded and only the genetical and cytological aspects of sex determination are dealt with, though without illustrations. Omissions are unavoidable in a book of this size, but the reviewer would have included such topics as sex determination in *Culex*, partial sex linkage and sex determination in hybrids. The volume has a definite zoological bias. There are a few misprints. H. KALMUS

THE ASSOCIATION OF APPLIED BIOLOGISTS

ANNALS OF APPLIED BIOLOGY, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1953

Dr.

EXPENDITURE

	£	s.	d.	£	s.	d.
To Estimated Value of Stock, 1 January 1953				189	18	0
To Cambridge University Press:						
Paper and Printing	2616	12	9			
Binding	282	8	10			
Carriage	116	5	7			
Insurance and Sundries	25	6	5			
Commissions	675	2	9			
				3715	16	4
To Preparation of Index				10	10	0
				£3916	4	4

Cr.

INCOME

	£	s.	d.	£	s.	d.
By Sales—Current volume	2355	15	0			
Back volumes, parts and sets	278	3	10			
Reprints and Advertisements	269	11	5			
				2903	10	3
By Estimated value of Stock—31 December 1953				214	7	0
By Balance carried down				798	7	1
				£3916	4	4

GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1953

Dr.

EXPENDITURE

	£	s.	d.
To *Annals of Applied Biology*, balance brought down	798	7	1
To Printing and Stationery	59	3	1
To Postages and cheque stamps	67	0	7
To Subscription—Parliamentary Science Committee	7	7	0
To Donation—Biological Council	2	0	0
To Sundry out-of-pocket expenses of Editors, Secretaries and Treasurer	16	3	6
To Refreshments and Hire of Rooms	13	7	3
To Audit Fee	7	7	0
To Balance being excess of Income over expenditure for the year	165	12	1
	£1136	7	7

Cr.

INCOME

	£	s.	d.	£	s.	d.
By Members' Subscriptions:						
Arrears	23	0	0			
Current	1013	12	6			
				1036	12	6
By Proceeds, List of Common Names:						
Part 1	6	4	0			
Part 2	10	7	0			
				16	11	0
By Interest received and receivable:						
2½% Defence Bonds	10	0	0			
National Savings Certificates	47	18	9			
Post Office Savings Bank	25	5	4			
				83	4	1
				£1136	7	7

BALANCE SHEET AT 31 DECEMBER 1953

Dr.

LIABILITIES AND SURPLUS	£	s.	d.	£	s.	d.
Sundry Creditors and Accrued charges:						
Cambridge University Press	902	6	1			
Audit Fee	7	7	0	909	13	1
Subscriptions paid in advance				80	12	0
Life Membership Fund Reserve:						
As at 1 January, 1953	200	0	0			
Addition during year	50	0	0	250	0	0
Excess of Assets over Liabilities:						
As at 1 January 1953	2849	14	2			
Add: Royal Society Grant	300	0	0			
Special Grant	45	0	0			
Excess of Income over Expenditure for the year 1953	165	12	1			
	3360	6	3			
Less: Transfer to Life Membership Fund	50	0	0	3310	6	3
				4550	11	4
Jubilee Fund:						
Balance as at 1 January 1953	110	18	0			
Donations (7) received during the year	33	5	0			
Bank Interest	2	6	1	146	9	1
				£4697	0	5

Cr.

ASSETS	£	s.	d.	£	s.	d.
Cash:						
Westminster Bank—Current Account	596	17	9			
Post Office Savings Bank Account	1169	14	1	1766	11	10
Amount Receivable from Royal Society				300	0	0
2000 National Savings Certificates (Cost £1265. 1s. 0d.)				1869	12	6
2½% Defence Bonds				400	0	0
Stock of *Annals of Applied Biology* as estimated				214	7	0
				4550	11	4
Jubilee Fund—Balance at Westminster Bank				146	9	1
				£4697	0	5

H. F. BARNES, *Hon. Treasurer*

I certify that the foregoing accounts are properly drawn up in accordance with the books, vouchers and documents produced to me, and in my opinion the Balance Sheet exhibits a true and correct view of the state of affairs of the Association according to such book, vouchers and documents.

(Signed)
J. B. BENNETT
Chartered Accountant } *Auditor*

HARPENDEN, 1 March 1954

Vol. 41, No. 3 September 1954

THE ANNALS OF APPLIED BIOLOGY

EDITED FOR THE ASSOCIATION OF APPLIED BIOLOGISTS

BY

R. W. MARSH

AND

I. THOMAS

WITH THE ASSISTANCE OF

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THE EFFECT OF TIME OF APPLICATION OF NITROGENOUS FERTILIZER ON POWDERY MILDEW OF WINTER WHEAT

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(With 4 Text-figures)

Applying nitrogenous fertilizer (N) to wheat plants of different ages affected the incidence of powdery mildew, *Erysiphe graminis* DC, differently. When N was applied before the flag leaf had emerged, the infection-index (number of pustules per 100 sq.cm. of leaf blade) increased to a maximum and then declined. If N was applied after the flag leaf had emerged, the infection-index increased steadily without reaching a peak, and the increased susceptibility was not associated with an increased relative leaf growth rate as when N was applied earlier.

The integral of the number of pustules per unit area with time, and the number of perithecia on the leaves of the main stem, both increased with increasing delay in the application of N. Plants given N in either April or May had at least three times as many pustules as those given N in January. The time when N was applied did not affect the date when perithecia appeared.

After adding N to nitrogen-deficient plants, the already mature leaves which had resisted mildew infection, became susceptible. This change is not associated with changes in the epidermal wall.

Introduction

The incidence of wheat powdery mildew, caused by *Erysiphe graminis* DC, depends on the nitrogen supply of the host and on the temperature (Last, 1953). The integral of the number of pustules per unit area with time increases with increasing amounts of nitrogenous fertilizer applied to the plants and with increases in the average relative leaf growth rate. Increasing the temperature from 7° to 14° or 20° C. increases host susceptibility, the rate of growth of the fungus and the rate of conidia production.

The outbreaks of cereal powdery mildew in field crops largely reflect the way in which temperature and fertilizers interact to determine susceptibility. This paper describes two pot experiments made with wheat to elucidate this interaction, and shows that plants given nitrogenous fertilizer before the flag leaf has emerged behave differently from those receiving nitrogen later.

Experiment I

Nitrogen given before the emergence of the flag leaf

Six grains of winter wheat, variety Red Standard (Squareheads type), treated with 'Ceresan', were sown in each of thirty-two pots (7 in. Sherdley pots) on 30 October

1950, and the seedlings were later reduced to five per pot. Each pot contained 3·6 kg. of 3:1 mixture by weight of washed sand and an infertile soil taken from Bones Close field on Rothamsted Experimental farm, and was given 2·0 g. K_2SO_4 and 1·5 g. $Ca(H_2PO_4)_2H_2O$ in solution before the wheat was sown. One series of pots (series Y) received 1 unit of nitrogenous fertilizer and a second (series X) none at sowing. The unit of nitrogenous fertilizer (N) was 0·3 g. $NaNO_3$ plus 0·4 g. $Ca(NO_3)_2 4H_2O$ applied in solution. Subsequently 4 units of N were given to different pots in series Y and 5 units to different pots in series X on three dates so the pots in both series, X and Y, received a total of 5 units of N. T_1, T_2 and T_3 denote treatments, which were replicated four times, in which N was given on 20 January, 10 April or 8 May 1951, respectively. Four additional pots in each series received no further N after sowing: these are called T_0. The replicate pots of each treatment were arranged in four randomized blocks in a well-ventilated and unheated greenhouse.

Observations of growth

The successive changes in the relative leaf growth rates (RLGR) of plants receiving the various treatments are plotted in Fig. 1. RLGR is given by the formula

$$\mathrm{RLGR}=\frac{\log_e a_2-\log_e a_1}{t_2-t_1} \quad \text{(Gregory, 1921),}$$

where a_1 and a_2 are the leaf areas in sq. cm. at dates t_1 and t_2 respectively. A difference between means of 0·012 is significant at the 5% level.

Differences between plants were first observed in late February, when in pots without added nitrogen the oldest leaves yellowed and no new shoots were produced. As all plants grew until then at the same rate, Fig. 1 records only data from February onwards.

With T_0 a higher RLGR was maintained by plants in series Y than in series X during February and March, +0·04 against +0·015. Subsequently, although the plants in series Y remained larger than in series X, their growth rates did not differ appreciably from +0·01.

Plants in series X and Y given treatment T_1 had the same RLGR. It reached a maximum of +0·06 at the end of March, significantly higher than that of T_0 in either series, and then declined to +0·01 which was maintained from the end of April to June.

After N was given in T_2 and T_3, the plants in series X gave significantly higher values of RLGR than those in series Y. Until then their growth was the same as in their respective T_0. The maxima were reached during late April and early May and were +0·083 and +0·046 for T_2 in series X and Y respectively. For T_3 the maxima occurred during late May and were +0·062 and +0·042 for series X and Y respectively.

All plants had their greatest leaf area immediately before ear-emergence, which started on 4 June except in T_0, T_2 and T_3 in series X (with these treatments the latest date was 12 June). The maximum leaf areas, including leaf blades and sheaths, which are given in Table 1, show that nitrogen given after sowing significantly increased leaf area, and that, except for T_1, plants in series Y were larger than in series X.

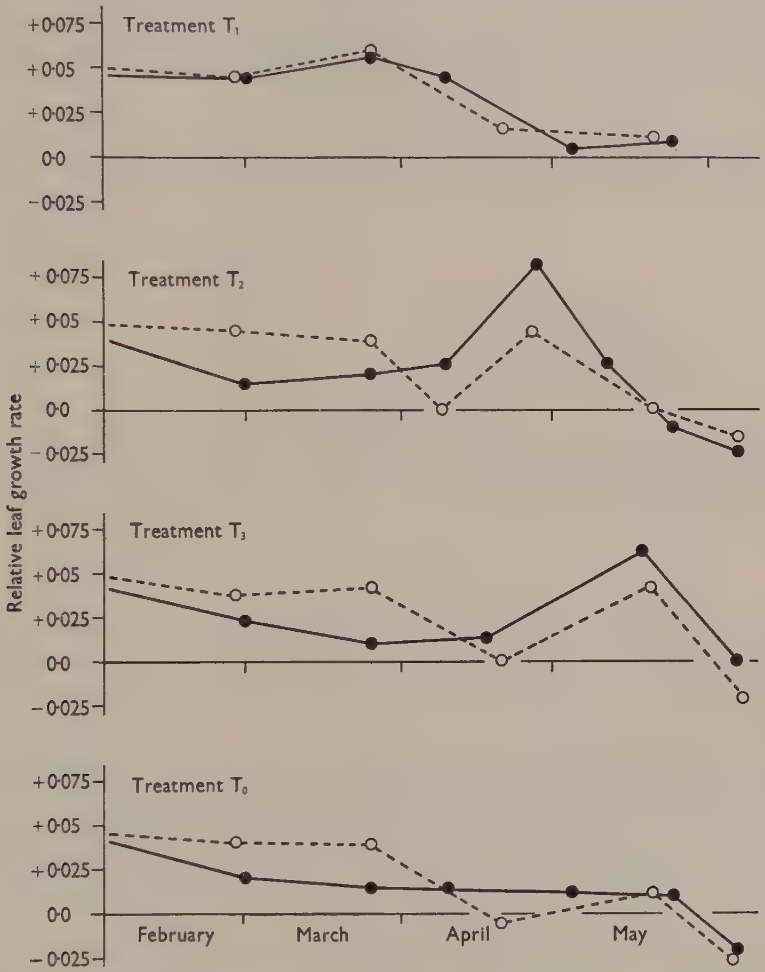

Fig. 1. Exp. 1. Changes with time of relative leaf growth rates. ●——● = series X (0N at sowing + 5N later); o- - - - o = series Y (1N at sowing +4N later). 0N, 4N and 5N represent 0, 4 and 5 units of nitrogenous fertilizer; 4N or 5N were given on 20 January, 10 April and 8 May in treatments T_1, T_2 and T_3 respectively.

The largest plants, which were in T_1, maintained a leaf area greater than 819 sq.cm. from 20 April, while with T_2 and T_3 the leaf area significantly increased above that of T_0 only after the addition of N. The leaf areas of the plants in T_0, 111 and 253 sq.cm. for series X and Y respectively on 20 April, steadily increased to the maxima. The differences between plants in series X and Y given T_2 and T_3 may be due to the effect of disease—with both T_2 and T_3, plants in series X had smaller maximum leaf areas and were more diseased than those in series Y. The straightforward fertilizer effect on leaf area in the absence of mildew was not tested.

The growth response after adding nitrogen was partly attributable to an increased number of shoots per plant. The mean maximum number of shoots per plant were 8·2, 5·6 and 3·4 for T_1, T_2 and T_3 respectively. Most of these shoots soon died when the plants were severely diseased.

TABLE 1. *Effect of time of application of nitrogenous fertilizer (N) on the maximum leaf area (sq.cm.). 0N, 1N...5N represent units of N given*

		Series X 0N at sowing+5N later	Series Y 1N at sowing+4N later
T_0	Only N at sowing	179	291
T_1	4N or 5N given 20 January	944	948
T_2	4N or 5N given 10 April	600	706
T_3	4N or 5N given 8 May	466	765

Incidence of infection

The plants were naturally infected by air-borne inoculum. Disease was assessed by the method described by Last (1953). The infection-index is given by the formula:

$$\text{Infection-index} = \frac{y \times 100}{a_1 + a_2 + a_3} \ldots \text{ pustules per 100 sq.cm.}$$

where y = total number of pustules of the asexual stage of *E. graminis* on the leaf blades of the main stem with a total area (sq.cm.) of $a_1+a_2+a_3\ldots$. The total number of pustules is the sum of the geometric means of the replicates for each leaf position.

Pustules were first observed on 9 February and were randomly scattered among the plants, regardless of treatments. Infection occurred at least two weeks previously, for this is the minimum incubation period at the prevalent mean temperature of 3·5° C. (=38° F.) (unpublished data). By early March most of the plants were infected, but few had more than one pustule, usually on one of the oldest leaves.

The number of pustules increased rapidly, reaching a maximum of 31 per 100 sq.cm. at the end of March on plants given T_1—the only treatment in which the plants were not then nitrogen-deficient. These plants maintained a high RLGR of +0·05 throughout February and March, but there was no appreciable increase in the number of pustules until the end of March, although plants with this rate of growth are usually susceptible. In this experiment spread of the fungus was probably limited by low temperatures.

From the end of March the weather favoured spread and only the nutritional status of the plant governed the extent of disease. The curves showing successive changes of the infection-index of plants in all treatments are plotted in Fig. 2.

The curves for T_1, T_2 and T_3 are all similar in shape but differ greatly in absolute values. Each rises to a maximum and then declines. The maximum values of infection-index in both series are successively higher with increasing delay in applying N. Thus the maxima for T_1, T_2 and T_3 respectively are 30, 220 and 254 in series X and 30, 92 and 151 in series Y. Except for T_1 the maxima are greater in series X than in series Y. The differences in disease between series X and Y agree with the differences in RLGR, but there are no consistent differences in the

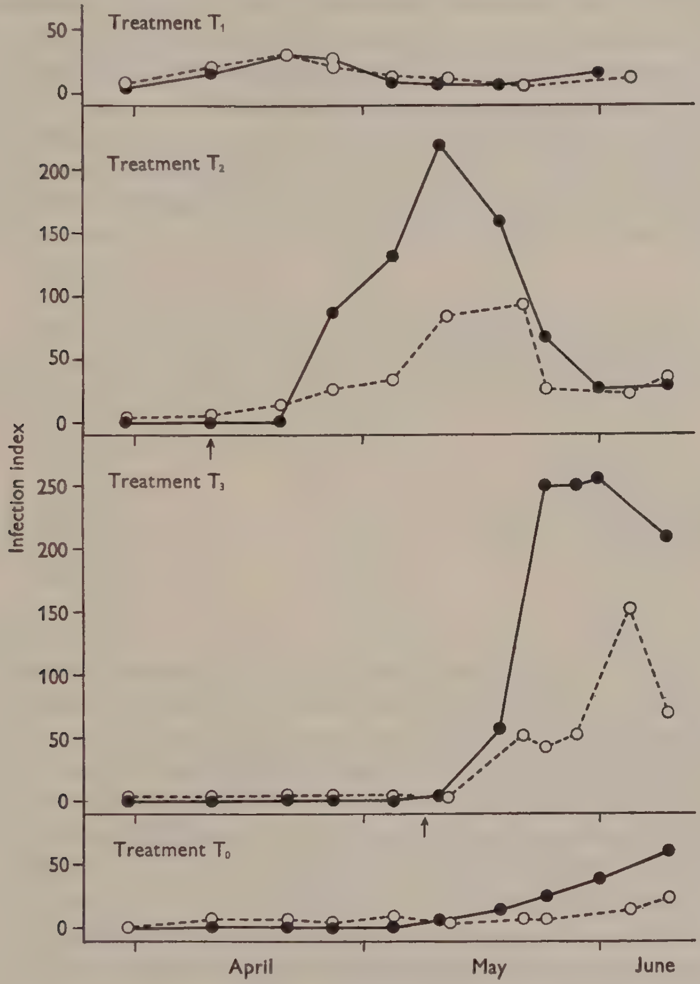

Fig. 2. Exp. I. Changes with time of the infection-index when nitrogenous fertilizer was given before the emergence of the flag leaf. ●——● = series X (0 N at sowing + 5 N later); o - - - - o = series Y (1 N at sowing + 4 N later). 0 N, 4 N and 5 N represent 0, 4 and 5 units of nitrogenous fertilizer; 4 N or 5 N were given on 20 January, 10 April and 8 May in treatments T_1, T_2 and T_3 respectively.

maximum RLGR of T_1, T_2 and T_3 to explain the time trend. After N was given in T_2 and T_3, the RLGR of the plants in series X was greater than in series Y. There was no difference between plants in series X and Y given T_1.

When the T_1 plants were most susceptible, there was little inoculum and they were little affected. By 10 April, when N was given in T_2 the inoculum was increased by the conidia from the infected T_1 plants and when N was given in T_3 (8 May) the T_2 plants were also providing conidia. Thus, even if the plants were equally susceptible after N had been given in treatments T_1, T_2 and T_3, differences would be expected because of the varying concentration of inoculum. The leaf sheaths did not die immediately after their complementary leaf blades, so although the leaf blade may have shrivelled its infected sheath still acted as a source of inoculum.

TABLE 2. *Effect of time of application of nitrogenous fertilizer (N) on the amount of disease (measured in mildew units).* 0*N*, 1*N*...5*N represent units of N given*

		Series X 0N at sowing+5N later	Series Y 1N at sowing+4N later	Mean
T_0	Only N at sowing	860	410	635
T_1	4N or 5N given 20 January	860	800	830
T_2	4N or 5N given 10 April	4970	2300	3635
T_3	4N or 5N given 8 May	4990	1940	3465
	Mean	2920	1365	—

Plants of treatment T_0 in series X became nitrogen-deficient in February. The oldest and infected leaves died and these plants did not become infected again until May. Subsequently the infection-index steadily increased to 59. In series Y the T_0 plants remained slightly diseased after the first infections appeared in February.

Until N was given, the plants in T_2 and T_3 resembled those in T_0 and resisted infection. Afterwards there was a lag of 6–11 days before susceptibility increased. The plants in T_2 resisted infection until mid-April and again in June but were susceptible in late April and May. In T_3 they were resistant until mid-May and then became susceptible and remained so. In T_1 the plants were susceptible in April and resistant in May and June. The areas under the infection-index curves of plants in all treatments are given in Table 2, where a mildew unit is one pustule per 100 sq.cm. for 1 day. These data sum the amount of disease from 30 March to 9 June.

Nitrogen given in January (T_1) had little effect on the amount of disease, whereas April (T_2) and May (T_3) applications increased it five to six times. Series X and Y did not differ with treatment T_1, but with T_2 and T_3 plants in series X had twice as many pustules as in series Y.

The amount of mildew on the ears was not measured; pustules were numerous only on plants in series X given T_3.

Perithecia (sexual stage) of *E. graminis* were first observed on 1 June when they were found on plants receiving all the treatments. In measuring their distribution (Table 3), each leaf blade and each leaf sheath on the main stem was examined separately and the number bearing perithecia was counted.

The development of the sexual stage, from initials invisible to the naked eye, to the visible black and spherical perithecia takes from 10 to 14 days (Cherewick, 1944). Therefore the perithecial initials were probably formed in mid-May, when the plants in T_2 and T_3 were bearing many pustules of the asexual stage, and those in T_0 and T_1 had only a few. Significantly more leaf blades and sheaths of plants in T_2 and T_3 bore perithecia than in T_0 and T_1 (5·3 and 7·3 against 1·8 and 1·3). There was no appreciable difference between plants in series X and Y. This assessment ignores the number of perithecia per blade or sheath but there were many more in T_2 and T_3 than in T_0 and T_1.

TABLE 3. *Effect of time of application of nitrogenous fertilizer (N) on the number of leaf sheaths and blades per main stem bearing perithecia. 0N, 1N...5N represent units of N given*

		Series X 0N at sowing+5N later	Series Y 1N at sowing+4N later	Mean
T_0	Only N at sowing	1·8	1·8	1·8
T_1	4N or 5N given 20 January	1·3	1·3	1·3
T_2	4N or 5N given 10 April	5·8	4·8	5·3
T_3	4N or 5N given 8 May	8·3	6·3	7·3
	Mean	4·3	3·6	—

Perithecia were formed on the ears only in T_3 of series X.

Addendum to Exp. I: Resistance mechanism. The changes from 30 March to 9 June in the level of infection of the individual leaves on the main stems of plants given treatments T_0 and T_2 in series X are plotted in Fig. 3. The levels of infection are given as the number of pustules per 100 sq.cm. of leaf blade area and the leaves are numbered so that leaf 6 is older than leaf 7 which is older than leaf 8 and so on. The data for leaf 5, which was the oldest leaf present on the main stem on 30 March, have been omitted as they are similar to those for leaves 6 and 7. Leaves 6 and 7 emerged on 1 March and 24 March respectively in both treatments. The dates when the other leaves emerged are indicated by the first point on each curve.

Until 10 April leaves 6 and 7 remained uninfected, but subsequently those in T_2 became very heavily infected after a lag of 10 days or more after receiving N. No macroscopic changes were detected in these already mature leaves, but the leaves emerging later had a greater surface area in T_2 than in T_0. The slopes of the infection curves for leaves 9, 10 and 11 (the flag leaf) in T_2 are progressively less steep and indicate a decline in susceptibility. There are no appreciable differences between the two treatments for leaves 10 and 11, showing that nitrogen no longer increased susceptibility.

From counts of the percentage germination of conidia on leaves of susceptible and resistant plants of the same variety Last concluded that, in the same environment, susceptibility was determined by host physiology. The data given in Fig. 3 support this conclusion, as the change in susceptibility of leaves 6 and 7, after adding N to plants in T_2, can only be due to changes within the host. Similarly,

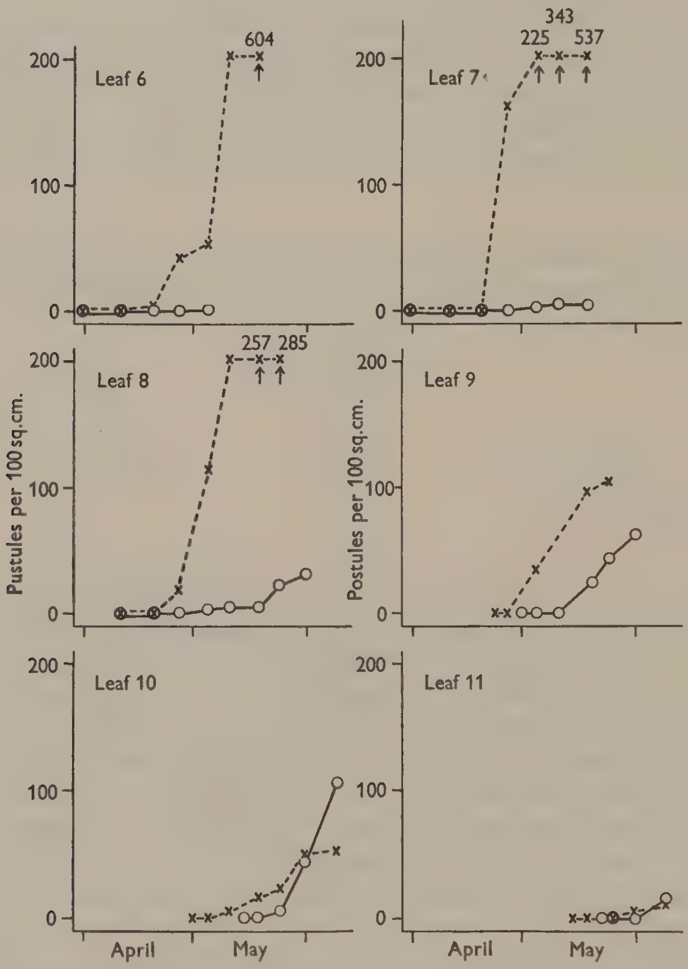

Fig. 3. Exp. I. Changes with time of the level of infection of individual leaves on the main stem of plants (*a*) given no nitrogeneous fertilizer (T_0) and (*b*) given nitrogenous fertilizer on 10 April (T_2). o——o $=T_0$, ×--× $=T_2$.

Corner (1935) found that in both resistant and susceptible wheat varieties germinating conidia of *E. graminis* produced infection papillae that reached the cytoplasm membrane, but then development ceased in the resistant variety, whereas haustoria were formed in the susceptible variety. If, as Sempio (1950) has suggested, susceptibility and resistance are determined by the balance between various physiological processes, then these processes in resistant varieties may be kept genetically in a balance similar to that of the resistant phase of a susceptible variety.

EXPERIMENT II

Nitrogen given after the emergence of the flag leaf

Six or sixteen grains of Red Standard wheat, as used in Exp. I, were sown in each of forty-eight pots on 12 December 1952 and later the number of seedlings was reduced to four or twelve per pot. Each pot contained 3·8 kg. of a mixture containing equal weights of air dried soil and sand, and was given 2·0 g. K_2SO_4 and 2·4 g. $Ca(H_2PO_4)_2H_2O$ soon after seedling emergence. Two and a half units of N were given to half of the pots of each sowing rate on 28 May 1953 after the flag leaf had emerged. The twelve replicate pots of each treatment were randomly arranged among plants bearing many pustules of *E. graminis*.

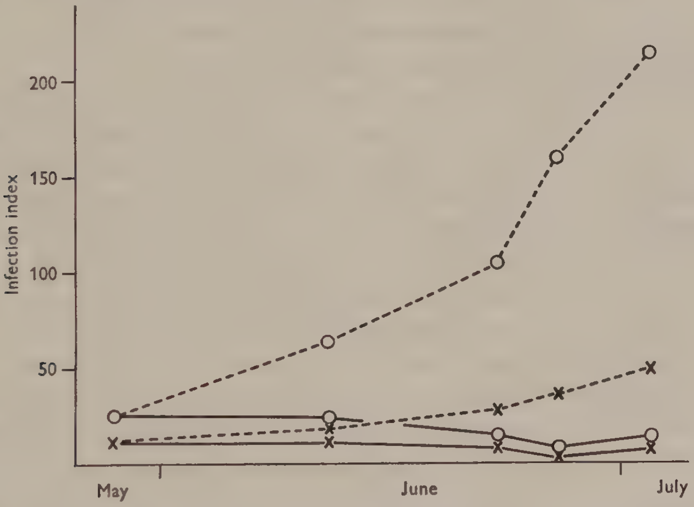

Fig. 4. Exp. II. Changes with time of the infection-index when nitrogenous fertilizer was given on 28 May after the emergence of the flag leaf. o = 12 plants per pot; × = 4 plants per pot; - - - N given, — no N.

When N was given each plant had only a single shoot; in pots containing only four plants, the mean leaf area of each plant was 281 ± 21 sq.cm. and in pots containing twelve it was 145 ± 22 sq.cm. The infection-index curves after applying N (Fig. 4) show that N progressively increased the infection-index, and that the initially smaller and more numerous plants (twelve plants per pot), became more heavily diseased than the initially larger plants (four plants per pot), although they received only one-third as much N per plant. This difference in severity of disease between treatments in which N was given is considered to reflect susceptibility differences, as the effect of varying plant number was nullified by surrounding the test plants with heavily infected plants. In addition, after N was given the difference in shoot number per pot was greatly reduced. Perithecia were formed about the same time on all plants. The addition of N increased the number of leaf blades and sheaths per plant that bore perithecia from 0·8 and 1·3 to 3·3 and 3·4 for four and twelve plants per pot respectively. The ears were infected in all treatments but perithecia were formed only on the twelve plants per pot receiving nitrogen—here 38% of the ears carried perithecia.

The differences in susceptibility between the two treatments receiving nitrogen cannot be related to corresponding changes in RLGR. Where there were four plants per pot, each plant produced two tillers after the addition of N. Where there were twelve plants per pot, there was an average increase of 0·7 tillers per plant with a range from nil to two. Thus, although there was no growth response by some of the twelve plants per pot, they all became more susceptible than the four plants per pot which gave consistent positive growth responses. The size of the existing leaves was not altered by the N added. Again, although some of the twelve plants per pot gave no growth response after the addition of N, they all became very heavily infected compared with those not given N.

The curves obtained after N was given in this experiment differ from those obtained in Exp. I, which followed a cycle showing an eventual change from a susceptible to a resistant phase. In that experiment the infection-index was continually reduced by exposing new and uninfected leaves, and when the plants reached the resistant phase the effect of these leaves was to reduce permanently the value of the infection-index. As the flag leaf (the last leaf to emerge on an ear-bearing shoot) had already emerged before the onset of the susceptible phase produced by N in Exp. II, the subsequent values of the infection-index were not reduced but steadily increased. There is, however, probably no difference in the internal factors determining susceptibility when N is given at different stages of growth. If the lives of the leaves could be prolonged sufficiently, then susceptibility would probably decline as when N is given before the flag leaf has emerged (Exp. I).

Discussion

The amounts of disease shown by plants with and without basal nitrogen (series X and Y) after the main fertilizer applications were made, in either April or May, are related to differences of RLGR (Exp. I). The higher the value of RLGR, the more diseased are the plants. This relation did not hold when nitrogen was given after ear-emergence (Exp. II). Then the less susceptible of the two sets of plants receiving fertilizer gave a more consistent and generally larger growth response than the more susceptible plants, some of which gave no growth response.

The number of tillers per plant produced when nitrogen was given in January, April or May, progressively decreased from a mean of 7·2 to 2·4 suggesting that this type of fertilizer response naturally decreases with increasing age of the plant.

As adding fertilizer did not increase the size of the existing leaves, but probably increased the size of the ears, the use of RLGR lacks precision as a measure of the metabolic rate, when the fertilizer is given after ear-emergence.

The experiments on the effect of time of applying nitrogenous fertilizer on the yield of wheat for which details of disease are not given, were reviewed by Lewis, Procter & Trevains (1938), and show that the yield of grain is increased equally whether the nitrogen is applied early (February–March) or late (April–May).

The yield of grain per plant depends on the number and the size of the ears. The final dry weight of each ear is derived from three sources, 25% is present at emergence, 30% is contributed by its own assimilation and the remaining 45% is given by the flag leaf and its sheath, the top internode and peduncle (Porter, Pal & Martin, 1950). Thus, when plants are not mildewed and the weather favours the development of the ear after emergence, the final size of each ear is determined by the absolute size of the initial 25%, which like the number of ears per plant, probably varies according to the effect of the previous conditions on the leaf area and net assimilation rate (N.A.R. is the dry matter gain by photosynthesis minus the loss by respiration). In England the N.A.R. of cereals follows a cycle closely related to the seasonal climatic cycle, and is lowest during the winter and highest in June (Watson, 1947).

The effect of mildew on the yield of cereals has not yet been studied sufficiently to determine its effect on the factors contributing to the final grain yield, but Schaller (1951) by comparing two wheat varieties, Atlas 46 (mildew resistant) and Atlas (mildew susceptible), found that infection reduced yield by 25%. Sempio showed that, within 14 days of infecting barley with *E. graminis*, the rate of photosynthesis was reduced and the rate of respiration increased. Both these changes must reduce N.A.R. and the total dry weight. Graf Marin (1934) found that within 21 days of infection the dry weight of barley was significantly reduced.

To measure the effect of mildew on yield, the effects of early seasonal infections on the number of ears per plant and on the absolute size of the ears at emergence still need to be measured, also the effects of late infections on these criteria and on the contributions made by the flag leaf and its sheath and the ear itself.

Late applications of nitrogen not only increased the number of pustules of the asexual stage of *E. graminis* on the leaves and ears but also increased the number of perithecia formed, and so increased the chances of the disease being 'carried over' from one season to another. Perithecia can mature and produce sexual spores (ascospores) when the host is dead, whereas asexual spores (conidia) are produced only on the living host.

These experiments indicate that nitrogen should not be applied late when there is a risk of mildew infection. The incidence of mildew was least increased when nitrogen was applied in January and it seems likely that the same result would be obtained as long as nitrogen is given before the plants become nitrogen deficient. A method of disease escape is to ensure that the plants make as much growth as possible while low temperatures prevent the rapid spread of disease. For winter cereals this means early application of fertilizers and for spring cereals it entails sowing as early as possible. In practice the solution of this problem is not so simple, and a compromise will probably have to be reached as these recommendations for decreasing mildew would create conditions that increase lodging caused by *Cercosporella herpotrichoides* Fron. (Salt, 1953).

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A STUDY OF THE EFFECT OF MILDEW INFECTION ON THE REACTION OF WHEAT VARIETIES TO BROWN RUST

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(With Plate 12 and 5 Text-figures)

The effect of the presence of *Erysiphe graminis tritici* on the reactions of each of five wheats, Malakoff, Democrat, Mediterranean, Hussar and Webster, to *Puccinia triticina* was studied. Under normal environmental conditions, mildew increased the susceptibility to rust of Malakoff and Democrat, though not, except in isolated instances, that of Mediterranean or Hussar. High light intensities enhanced the effect of mildew on the reaction of Democrat to *P. triticina*. The susceptibility of Webster to rust was unaffected or decreased by the presence of mildew.

In all varieties there was a decided tendency for rust and mildew to develop on different parts of the leaf, and when a leaf was heavily mildewed the development of *P. triticina* was sometimes almost entirely inhibited. The reason for this is obscure, since spores of *P. triticina* and *E. g. tritici* germinate well in each others presence, and cytological studies revealed several cases of the presence of both mildew and rust haustoria in a single living host cell. Anatomical investigations also showed that the effect of mildew on rust infection was confined to rust pustules within 1 mm. of mildew colonies.

The results obtained are in accordance with Gassner's theory that susceptibility to rust is due to the presence of certain specific proteins in the host leaf.

Introduction

Johnston (1934) found that occasional large uredinia developed on wheat plants of a variety normally resistant to the physiologic race of *Puccinia triticina* Erikss. with which they had been inoculated. These large uredinia were associated with infections of *Erysiphe graminis tritici* DC. Mains (1933) also noticed that large uredinia developed on the leaves of a normally resistant variety when mildew was present. Roberts (1936) found pustules of a susceptible type developing near mildew colonies on normally resistant wheats. This was most marked in the variety Malakoff, but was also seen in Democrat and Webster.

The purpose of the present study has been to investigate systematically the relationship between the two parasites when inoculated on to a single wheat leaf under various conditions.

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* Now at the Mushroom Research Station, Yaxley, Peterborough. The practical work described in this paper forms part of a thesis by D. G. Gandy, accepted for the Degree of M.Sc. by the University of London.

INFECTION EXPERIMENTS

Experimental methods

Stock cultures were maintained on Wilma, a wheat variety susceptible to both rust and mildew. The experimental methods used for the establishment and maintenance of cultures were those described by Manners (1950). The stock culture of *P. triticina* was a single spore culture of race 66 isolated by Roberts (1936) and maintained at the Botany School, Cambridge, who kindly supplied it. It had remained constant and still gave the reactions of race 66 (see Table 1). The stock culture of *E. g. tritici* was obtained by making a single spore culture from a field collection, using a modification of the dry needle method of Mains & Jackson (1926). The seed of the differential hosts for *P. triticina* was derived from that originally used by Mains & Jackson (*loc. cit.*).

TABLE 1. *The reactions of the differential hosts to* P. triticina, *race* 66

	(*a*) In 1950	(*b*) According to Roberts (1936)
Malakoff C.I. 4898	0–1	0
Loros C.I. 3779	3–4	4
Democrat C.I. 3384	0–1	0–1
Mediterranean C.I. 3332	0–2	0–1
Carina C.I. 3756	2–3	3–4
Brevit C.I. 3778	3–4	4
Hussar	2–3	4
Webster C.I. 3780	0–1	2–3
Norka C.I. 4377	0–1	—

The wheats chosen for further study were Malakoff, Democrat, Mediterranean, Hussar and Webster. These varieties are all susceptible to the culture of *E. g. tritici* employed, and semi-resistant to race 66 of *P. triticina*: it is apparent from previous work that semi-resistant rust reactions are particularly liable to modification by external agencies. Experiments were carried out in each of four environments:

'N', 21° C. with daylight only,
'L', 21° C. with daylight and artificial light,
'T', 27° C. with daylight only,
'LT', 27° C. with daylight and artificial light.

The required temperatures were maintained by thermostatically controlled greenhouse heating, but in warm weather it was impossible to maintain a temperature of 21° C., the optimum temperature for the development of *P. triticina*. By the use of blinds, free ventilation and frequent syringing, the temperature in the cooler section was, however, kept below 27° C. Artificial light was supplied by fluorescent tubes giving an intensity of 100 f.c. at 21° C., but only 60 f.c. at 27° C., owing to the shading effect of the frame in which the higher temperature was maintained.

The assessment of the results

The reaction type, giving a measure of the degree of resistance shown to rust, was recorded on the scale proposed by Mains & Jackson (1926). The intensity of attack was assessed by estimating the percentage area of the leaf covered by rust pustules, with the aid of standard area diagrams. It was found, as Last (1953) noted, that the distribution of mildewed areas on a leaf did not correspond with that shown in the standard diagrams made by Grainger (1947), so a fresh set was prepared, together with a new set for the assessment of brown rust intensity (see Text-figs. 1 and 2). In the case of both parasites, the intensity of attack depends on the amount of inoculum employed, so care was taken to standardize this as far as possible. Owing to spontaneous secondary infections, *E. g. tritici* continues to spread indefinitely, and the percentage area covered depends on the time which has elapsed since inoculation. All assessments were therefore made 8–12 days after inoculation, depending on the time of year, by which time both rust and mildew were well developed.

In all cases the upper surface of the leaf only was inoculated, but in the case of rust, pustules usually developed on both surfaces and the total rust intensity was expressed as the mean of the values recorded for each surface. Mildew seldom spread to the lower surface within 12 days, so the upper surface only was assessed. In the later experiments the percentage of the leaf area showing chlorosis or necrosis was also estimated.

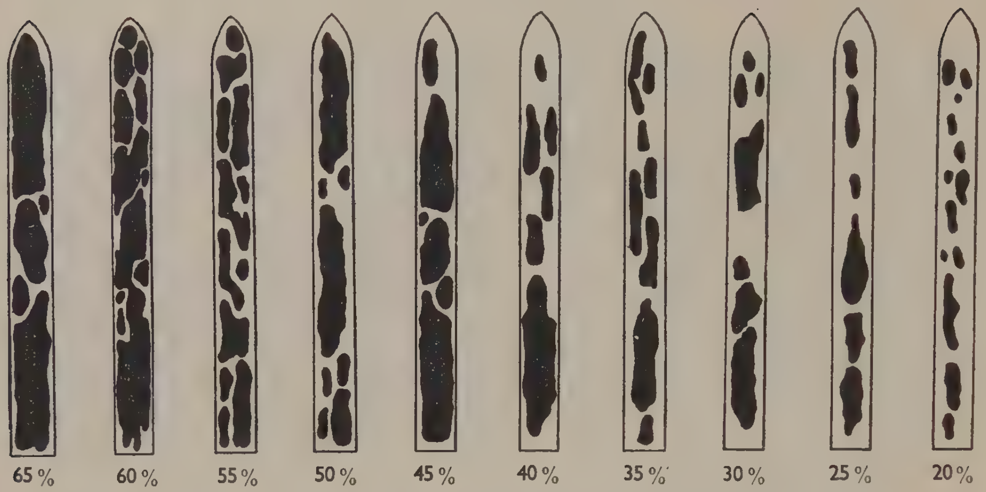
Results

Five experiments were carried out. In each experiment five pots (each containing three plants) of each of the five varieties employed were placed in each of the four environments. The five pots were inoculated as follows:

I Control, *P. triticina* only.
II Control, *E. g. tritici* only.
III *P. triticina* and *E. g. tritici* simultaneously.
IV *P. triticina* 48 hr. before *E. g. tritici*
V *P. triticina* 48 hr. after *E. g. tritici*.

(*a*) *The results of simultaneous rust and mildew inoculation at normal temperatures* (21° C.) *in daylight*

These results are given in Table 2. In Malakoff the normal reaction to rust was 0–1, except at the high temperatures and light intensities prevailing during Expt. A (August 1950) when it rose to 2–3. The reaction type was increased from 0 or 0–1 to 1 in Exps. B (October 1950) and D (February 1951) and in Exps. C (November 1950) and E (April 1951) necrosis was more widespread in the presence of mildew, indicating a greater spread of rust mycelium before necrosis ensued. The presence of mildew caused a slight increase in the number of rust pustules in all but one of the experiments with Democrat, and in that experiment pustules of a more susceptible


65 %
60 %
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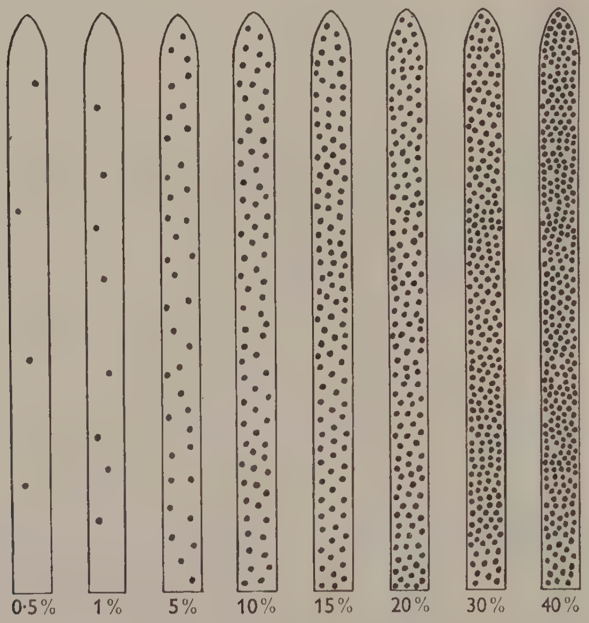
Text-fig. 1. Provisional standard diagrams for the estimation of intensity of mildew attack.


0·5 %
1 %
5 %
10 %
15 %
20 %
30 %
40 %

Text-fig. 2. Provisional standard diagrams for the estimation of brown rust attack.

TABLE 2. *Effect of simultaneous mildew inoculation on the results of rust inoculation at 21° C. in daylight*

Exp. ...	A			B			C				D				E			
	Reaction type	% *P.t.*	% *E.g.*	Reaction type	% *P.t.*	% *E.g.*	Reaction type	% chlorosis	% *P.t.*	% *E.g.*	Reaction type	% chlorosis	% *P.t.*	% *E.g.*	Reaction type	% chlorosis	% *P.t.*	% *E.g.*
Malakoff, I, II	3	8	33·3	0	0	50	0	0	0	20	0–1	11·7	3	45	i	0	0	65
Malakoff III	2–3	6	33·3	1	5	40	0	5·5	0	21·7	1	?	5	40	0	5	0	50
Democrat I, II	—	—	—	0–1	0·1	60	0–1	13·3	1	20	1	50	13·6	50	0–1	25	3	65
Democrat III	—	—	—	0–1	6·3	50	0–1+	10	0·5	13·3	1+	45	15	0	0–1	23	4	75
Mediterranean I, II	1	4	55	0–1	7	56·7	0	5	0	20	1	5	2·3	30	1–2	30	16·5	30
Mediterranean III	1	3·5	58·3	1	7·2	50	0	7·5	0	33·3	1–2	0	5	33·3	2	13	11·5	40
Hussar I, II	3	28·8	60	1–2+	15	50	0	10	5	23	1	60	2·2	50	2	0	12·5	62
Hussar III	3	15·7	21·7	1–2	10·8	50	1	?	5	20	2	0	4·5	45	2	1	3·5	55
Webster I, II	2	3·8	52·5	0+	0·1	20	0	5	0·1	10	1	0	0·1	60	1–	10	10	65
Webster III	1–2	9	45	0	0	66·6	0	5	0	35	i	0	0	70	0–1	18	2·5	33

I, II denote rust and mildew inoculations on separate plants; III denotes simultaneous inoculation on the same plant. *P.t.*=*P. triticina*; *E.g.*=*E. g. tritici.*

TABLE 3. *Effect of mildew attack on the distribution of* P. triticina *on the leaf*

Exp. E...	I. *P. triticina* alone		III. *P. triticina*+*E. g. tritici*		
	% rust		% rust		
	Upper surface	Lower surface	Upper surface	Lower surface	% mildew
Democrat	5	1	4	4	75
Mediterranean	18	15	10	13	40
Hussar	10	15	1	8	55
Webster	10	10	1	4	33

type than usual were noted around the mildew infections. In Mediterranean and Hussar no significant change in reaction type or number of rust pustules was caused by mildew infection. The presence of mildew on Webster decreased the susceptibility to rust both as regards reaction type and number of pustules. This result fails to confirm the findings of Roberts (1936) who found that Webster, like Malakoff and Democrat, exhibited enhanced rust susceptibility in the presence of mildew, and will be discussed later.

Any increase in susceptibility was usually limited to pustules in close proximity to *E. g. tritici* colonies. There was a general tendency for rust and mildew colonies to occupy different parts of the leaf: when the upper surface was heavily mildewed, rust pustules were to a large extent confined to the lower surface, or were at any rate more abundant there than on the upper surface (Table 3).

(*b*) *The effect of different temperatures and light intensities on infection by* P. triticina

Both the environmental differences between the four series N, L, T and LT (see p. 394) and those between the experiments due to the various times of year at which they were made, must be considered. Exp. A was carried out in August 1950, when the natural light intensity and temperature were both high, and there was little difference between the four environments. The susceptible reaction of Malakoff in this experiment was not reproduced in any later one. Exps. B, C and D were carried out in October 1950, November 1950 and February 1951 respectively, when external temperatures and light intensities were low and the effects of the different environments were revealed. In Exp. E, carried out in April 1951, when the light intensity was fairly good, artificial light scarcely affected the results, but increasing the experimental temperature lowered the susceptibility of several varieties.

When *P. triticina* alone was inoculated, increased light increased both the number of pustules and the amount of chlorosis, often leaving the reaction type almost unchanged. The reactions of Malakoff and Webster were rendered rather more susceptible by high light intensities at 21°, but not at 27°. Increased temperatures usually rendered Malakoff immune, but decreased both the number of pustules and the amount of chlorosis in Mediterranean, Democrat and Hussar. Hussar being inherently more susceptible than the other varieties employed, increased light increased the number of pustules, but not the amount of chlorosis, and so altered the reaction type. Many of these effects are illustrated in the results obtained in Exp. D (Table 4) and Exp. B (Pl. 12).

The development of *E. g. tritici* was not clearly correlated with temperature or light intensity.

(*c*) *The effect of different temperatures and light intensities on the interaction between* P. triticina *and* E. g. tritici

The somewhat increased susceptibility of Malakoff to rust in the presence of mildew was maintained under all conditions of light and temperature employed. The slight increase in the susceptibility of Democrat to rust seen in the presence of mildew was particularly marked at high light intensities, where, in the presence of mildew, the number of pustules was greater than that in the controls; there being no corresponding increase in the amount of chlorosis, the reaction type was rendered more susceptible than that of the control plants. Whatever the environmental conditions, the presence of mildew caused no consistent increase in the susceptibility of Mediterranean or Hussar to *P. triticina*. In Webster, at high light intensities, as at normal ones, there was rather less rust on mildewed than on non-mildewed plants. In normal light at high temperatures Webster is decidedly resistant to *P. triticina*, whether mildew is present or not. Certain of these results are illustrated in Table 4.

TABLE 4. *Effect of environmental factors on the susceptibility of wheats to attack by* P. triticina *alone and in the presence of* E. g. tritici

		Rust reaction type		% chlorosis		% rust intensity	
Exp. D	Environment	I	III	I	III	I	III
Malakoff	N	0–1	1	11·7	0	3	5
	L	1	1	20	5	7·5	0·9
	T	i	1	0	0	0	0·3
	LT	1	1+	10	0	1	0·7
Democrat	N	1	1+	50	45	13·6	15
	L	1	2–3	20	12·5	15	14·2
	T	1	1–2	0	0	2·5	2·5
	LT	0–1	1	0	30	7·5	10·5
Mediterranean	N	1	1–2	5	0	2·3	5
	L	1	2–3	30	5	12·5	7·5
	T	i	1–2	0	0	0	3·8
	LT	1–2	2	10	4	4·5	9·9
Hussar	N	1	2	60	0	2·2	4·5
	L	2+	3	22·7	0	10·8	7·5
	T	1	2–3	0	15	0·5	8·2
	LT	2+	2+	0	0	7	3·4
Webster	N	1	i	0	0	0·1	0
	L	1	i	0	0	1	0
	T	i	0–1	0	5	0	1
	LT	1	1	5	0	1	2

I denotes inoculation with *P. triticina* alone, III inoculation with both *P. triticina* and *E. g. tritici* simultaneously.

(*d*) *The effect of inoculation with* P. triticina *and* E. g. tritici *at different times*

In set IV the plants were inoculated with mildew 48 hr. after the rust inoculation, in set V, 48 hr. before. The results of these experiments were not sufficiently consistent for it to be possible to draw any detailed conclusions, but it was frequently noted that when, in plants inoculated with mildew previous to the rust inoculation, mildew developed rapidly, rust failed to develop. Cytological examination of such leaves revealed no rust mycelium in the mesophyll. This suggested that germinating mildew conidia might inhibit the germination of rust conidia. However, when rust and mildew spores were germinated together either on wheat leaves or on damp 'Cellophane', germination of both types of spore was good, and an explanation for the inhibition of *P. triticina* by *E. g. tritici* under certain conditions must be sought elsewhere.

ANATOMICAL INVESTIGATIONS

Allen (1926, 1927) studied the development of *P. triticina* race 11 on Little Club (fully susceptible) and Malakoff (resistant). The process of host penetration by *E. graminis* was described by Smith (1900) and Corner (1935).

Methods

Material was taken from inoculated plants when the rust had developed sufficiently for the reaction type to be determined, about 8–12 days after inoculation. Material was fixed in Schaffner's chromo-acetic fixative, embedded and cut, usually in L.S., at 10–12μ. Thinner sections contained only short pieces of mycelium. Sections were stained in Heidenhain's Haematoxylin or Flemming's Triple Stain, the latter being the more satisfactory of the two.

Results

(*a*) *Inoculations with* P. triticina

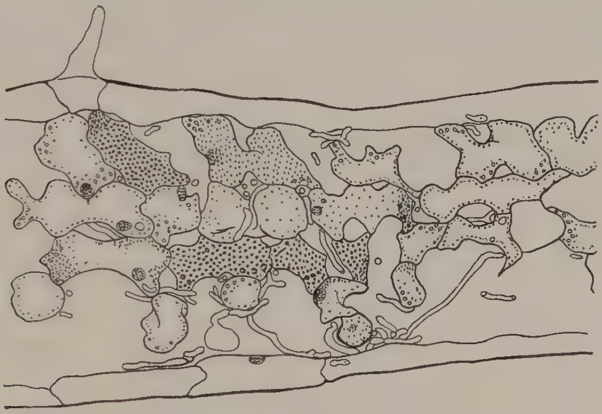
The reaction types recognized by Mains & Jackson (1926) proved reliable guides to the anatomical structure of tissues infected with *P. triticina*. Sections of leaves exhibiting a type 0 (highly resistant) reaction contained patches of mesophyll tissue which stained bright red with the Triple Stain. The cell contents of such tissue were disorganized and the cell walls had frequently collapsed. Such tissue, first described by Allen (1927) for the reaction of Malakoff to race 11 was observed in Democrat. The reaction of a resistant host was sometimes less violent, for example in Mediterranean, where rust growth was slow, the mycelium appeared stunted and misshapen, and disintegrating host tissue was found wherever chlorotic flecking occurred. In more susceptible hosts, e.g. Hussar, damage was limited to a slight thickening of a few mesophyll cell walls.

(*b*) *Inoculations with* E. g. tritici

The wheat varieties employed were highly susceptible to *E. g. tritici* and the fungus developed freely. Epidermal cells were frequently found with three or four haustoria inside, yet the nuclei of such cells appeared to suffer no damage.

(*c*) *Double inoculations*

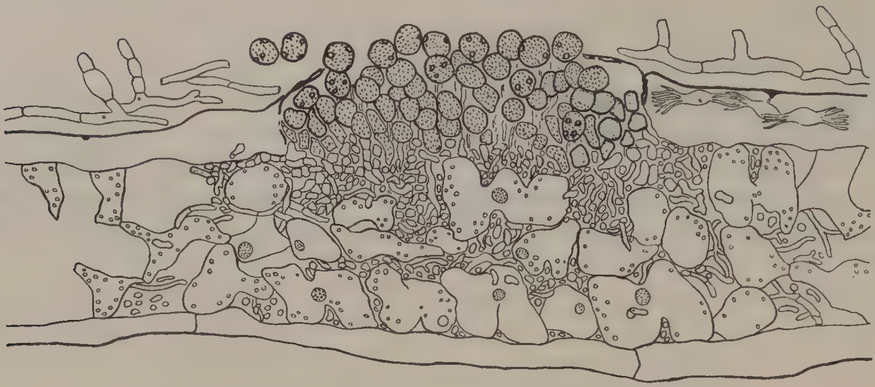
A large number of doubly inoculated leaves was examined, but the very small number which showed increased susceptibility to *P. triticina* in the presence of *E. g. tritici* demonstrated that the mechanism causing this increase was very local in its effect. Pustules developing more than 1 mm. away from a mildew colony were unaffected (Text-fig. 3). Increased susceptibility generally occurred only when a mildew colony developed immediately above a rust colony and was characterized by a partial or total absence of dead or damaged mesophyll cells at the centre of the rust infection (Text-fig. 4).



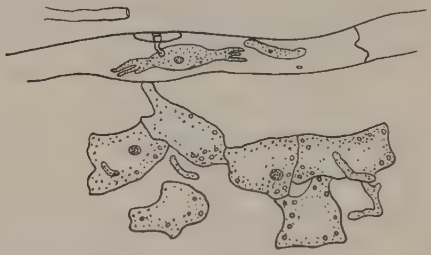
Text-fig. 3. L.S. leaf of Democrat showing a resistant reaction to *P. triticina* in the absence of *E. g. tritici.* ×275.

Owing to their different growth habits, it was not possible to find the mycelia of both pathogens occupying the same tissue. The closest contact between them was found in the leaf epidermis where both rust and mildew haustoria were found in the same cell. Both appeared normal and the host nucleus was unaffected (Text-fig. 5).

The phenomenon of ‘green islands’ was frequently noted on leaves which had otherwise become yellow. These islands could be caused by the presence of either pathogen. They have been variously interpreted as a tendency towards symbiosis (von Tubeuf, 1897), a result of nitrogen fixation (d’Oliviera, 1939) or the result of the pathogen having obtained nourishment from the more distant parts of the leaf first. Cells in green islands appeared to contain more chloroplasts than those elsewhere, but the increase was not statistically significant. The green colour of the islands must therefore be largely due to the individual chloroplasts having a high chlorophyll content.



Text-fig. 4. L.S. leaf of Democrat showing a susceptible reaction to *P. triticina* in the presence of *E. g. tritici*. ×275.



Text-fig. 5. L.S. leaf of Democrat showing both rust and mildew haustoria in a single epidermal cell. ×275.

DISCUSSION

Johnston (1934) suggested that the increased susceptibility of a wheat variety to *P. triticina* in the presence of *E. g. tritici* was caused by the latter splitting complex compounds not available to the rust into simpler compounds which the rust could utilize. Increased susceptibility to *P. triticina* would then be expected to occur as frequently in highly resistant varieties as in semi-resistant ones, and this is not the case. If Johnston's view were correct, either the enzymes causing the breakdown of complex compounds, or the products of their activity would be expected to diffuse into the surrounding host tissue and the effect on *P. triticina* would be much less localized than it actually is.

Gassner and his co-workers came to the conclusion that susceptibility to rust fungi is based on the presence in a wheat variety of certain specific proteins.

Gassner & Franke (1934) considered that an increase in nitrogen nutrition increased the concentration of total protein in the plant, including that of these specific proteins, without altering the proportion of specific proteins. In very resistant wheat varieties the concentration of specific proteins is assumed to be low and any increase brought about by increased nitrogen nutrition would still not be sufficient to alter the host reaction. In semi-resistant varieties the concentration of these proteins is assumed to be sufficiently increased to alter the reaction type. Gassner & Hassebrauk (1938) increased the concentration of protein in wheat plants by treatment with chloroform vapour and susceptibility to *P. triticina* was correspondingly increased.

The photosynthetic tissue in green islands remains active for longer than that in surrounding tissues and the content of chlorophyll is exceptionally high. These two factors would cause local increases in the content of total protein and hence also of specific proteins. Since the green islands associated with the mycelium of *E. g. tritici* are larger than those of *P. triticina*, an explanation is provided for the increased susceptibility to *P. triticina* of tissues in close proximity to mildew colonies on semi-resistant wheats. In highly resistant varieties, the specific proteins concerned may be entirely absent, or present in such small amounts that the presence of mildew fails to increase the concentration sufficiently to alter the rust reaction. It is significant that increased susceptibility occurred most frequently and consistently on semi-resistant varieties such as Democrat and Mediterranean. It occurred on the more resistant wheats Malakoff and Webster only when plants were grown in good light, when conditions were presumably favourable for protein synthesis. It can therefore be concluded that the nature of the effect of the close proximity of colonies of *E. g. tritici* on infection by *P. triticina* is in accordance with the theory that specific proteins determine the susceptibility of wheat to rust attack.

We wish to thank Dr W. J. Dowson, of the Botany School, Cambridge, and Dr K. S. Quisenberry, of the U.S. Department of Agriculture, for supplying seed. Part of this investigation was carried out during the tenure of a University College, Southampton, Research Grant by D. G. Gandy.

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EXPLANATION OF PLATE 12

Fig. 1. Exp. B. Democrat. TI, *P. triticina* alone at 27° C. in daylight; TV, *E. g. tritici* inoculated 48 hr. before *P. triticina* at 27° C. in daylight; LI *P. triticina* alone at 21° C. in daylight + artificial light; LIII, *P. triticina* and *E. g. tritici* inoculated simultaneously at 21° C. in daylight + artifical light; LIV, *E. g. tritici* inoculated 48 hr. after *P. triticina* at 21° C. in daylight + artificial light.

Fig. 2. Exp. B. Hussar. LI, *P. triticina* alone at 21° C. in daylight + artificial light; LIII, *P. triticina* and *E. g. tritici* inoculated simultaneously at 21° C. in daylight + artificial light; LIV, *E. g. tritici* inoculated 48 hr. after *P. triticina* at 21° C. in daylight + artificial light.

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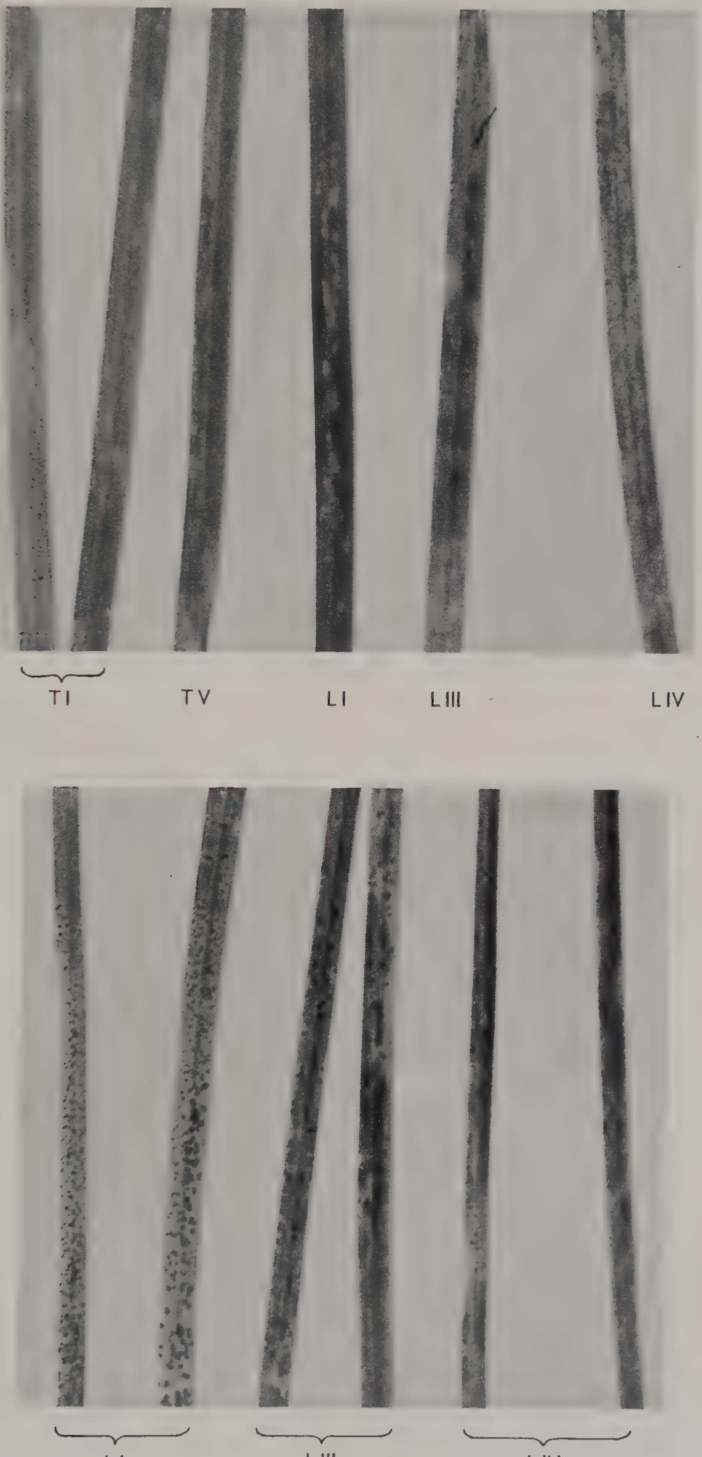
T I
T V
L I
L III
L IV
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MANNERS AND GANDY—*Effect of mildew on the reaction of wheat varieties to brown rust*

Williams, W. & Verma, U. N. (1954). *Ann. appl. Biol.* 41 (3), 405–416

INVESTIGATIONS ON RESISTANCE TO DISEASE AMONG SPECIES OF THE GENUS *AVENA*

I. RESISTANCE TO PHYSIOLOGIC RACES OF *USTILAGO AVENAE* AND *U. KOLLERI*

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Fifty-three samples representing species of the genus *Avena* were tested for resistance to infection by *Ustilago avenae* and *U. kolleri* following artificial inoculation. Among the diploid and tetraploid species tested, eleven out of thirty-seven samples of *Avena strigosa* subsp. *strigosa* showed complete resistance to all the cultures with which they were inoculated. *Avena strigosa* subsp. *barbata* (three samples), and *Avena strigosa* subsp. *abyssinica* (one sample), also proved to be resistant to all the available races.

Variation in morphological and physiological characteristics within species and samples (varieties?) of the lower chromosome groups of *Avena* were observed and its consequences in breeding and race identification discussed.

Race identification was carried out on the eight *Ustilago* cultures and the existence of at least six races established. The tester varieties used in the present study proved inadequate for the complete separation of the smut races.

Most of the cultivated varieties of oats belong to two hexaploid species of the genus *Avena*—*A. sativa* (L.) and *A. byzantina* (C. Koch), and plant breeding work has been concentrated on selection and hybridization within these two species. No successful attempt has been made to introduce genetic material from diploid and tetraploid species into our cultivated varieties, with the result that the majority of present day varieties stem from a very narrow genetic basis.

Improvement within one small group of the genus has had two consequences of far-reaching significance. First, several very good varieties of very high yielding capacity and high quality have been developed as a result of recombination and selection, while secondly, as a result of a reduction in genetic variability, many valuable genes for disease resistance have been discarded and lost from the material. Furthermore, when the development of new, improved varieties of cultivated oats is considered, it is noted that only a few varieties have contributed parental material for the wide range of oat varieties under cultivation in this country and elsewhere. Stanton (1936) observed that many of the improved varieties of oats in the U.S.A. were derived from Red Rustproof, Kherson, Sixtyday, Burt and a few others. More recently, the varieties Victoria and Bond have been extensively used for breeding. Likewise a large number of British and continental oat varieties have Swedish Victory as a common parent. The British varieties of oats having Victory as one of their parents include: Eagle, Golden Rain II, Maldwyn, Milford (S. 225), Primus, Star, Sun II, and S. 84. This list is not intended to be exhaustive but serves to illustrate that a large number of species and varieties of the genus *Avena* are excluded from the improvement programmes and that much of the variability has not been utilized. The importance of genetic diversity in plant breeding has only been recognized relatively recently, and the attempts that have been made to synthesize varieties from inter-specific hybrids are too few and too recent to allow a reliable assessment to be made of their ultimate value in crop improvement.

The danger inherent in breeding from depleted genetic diversity is clearly brought out in a report by Stevens & Scott (1950) who state that the average life of an improved variety of oats in the corn belt of the U.S.A. is from 4 to 5 years, after which time its resistance is broken by newly developed races of pathogens.

Although no experimental work is known where all the diploid and the tetraploid species of *Avena* have been tested for resistance, fairly good accounts are available on the resistance to several diseases of some diploid species. Vavilov, from extensive studies involving 350 pure lines from eight *Avena* species, reported that *A. strigosa* and *A. brevis* were partially resistant to crown rust, stem rust and to the smuts. Vavilov concluded that there are two groups—one susceptible, the other resistant, within diploid oats generally and particularly within *A. strigosa* and *A. brevis*. Malzew (1930) concluded that the general genetical behaviour of all the species towards diseases is more or less the same but that a greater degree of immunity to infection is shown by the sub-section Aristulatae which contains *A. strigosa*.*

MATERIAL AND METHOD

During the present study the reaction of different forms of diploid and tetraploid species of *Avena* to eight cultures of oat smuts was tested in the greenhouse and in the field. During the first year only two cultures of smut were available and these were designated L_3 and C_1.† In the following year, six more cultures of smut were included, and their reaction on identifier varieties as well as on the experimental samples was studied. All known details of smut cultures used in the present study are given below.

Designation	Source of origin	Species of smut
L_1	Isolated from the variety Anthony ex U.S.A.: September 1951	Loose smut (*Ustilago avenae*)
L_2	Isolated from sample no. 31: September 1951	
L_3	Isolated from variety (S. 147 (G.F. 50)) ex W.P.B.S. Aberystwyth: March 1951	
L_4	Isolated from the variety Muckle oat ex Orkney Islands: September 1951	
C_1	Isolated from sample no. 52: March 1951	Covered smut (*U. kolleri*)
C_2	Isolated from sample no. 35: September 1951	
C_3	Isolated from the variety R. 30 ex Aberdeen: September 1951	
C_4	Isolated from sample no. 34: September 1951	

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* *A. strigosa* Malz. comprises *A. brevis* Roth.

† As far as the authors are aware there is no connexion between the pathogen isolates used in the present work and those used in other studies and which may carry similar symbols.

Racial identification studies were conducted on the varieties of oats selected by Holton & Rodenhiser (1948) and kindly supplied by Dr T. R. Stanton, Beltsville, Maryland, U.S.A. The varieties used were the following: Anthony, Black Diamond, Victory, Gothland, Monarch, Black Mesdag, Camas, Lelina and Fulghum.

For the present study two methods of smut inoculations were adopted. Where only a small number of seeds were involved in the tests, spore dusting of de-hulled seeds was preferred, whereas for the field tests, involving a large number of seeds per sample, inoculation of surface sterilized seed by spore suspension under partial vacuum technique was suitable.

(1) *Spore dusting of de-hulled seeds*

In 1951, five to eight grains of each of the experimental samples were de-hulled and inoculated with large quantities of dry spores from the two cultures then available. The inoculated seeds were subsequently sown in pots and the plants matured in the greenhouse. No attempt was made during the first season to control temperature during the germinating period of the inoculated seeds.

During the second year (1952), twenty seeds of each of the experimental samples were treated in a manner similar to that already described, but the germinating temperature was controlled at 70° F. for 4–6 days. The seedlings were subsequently transplanted into the garden—each sample being represented by two rows of ten seedlings.

(2) *Inoculation by spore suspension under partial vacuum*

Twenty-four different seed samples were inoculated in 1951 by this method and the material was sown under field conditions. Seeds were spaced at one inch intervals in rows and replication was carried out wherever possible. In the second year two hundred seeds of each of twenty-two surface sterilized samples were inoculated with six different smut cultures, using the partial vacuum technique. The vacuum pressure and time of treatment used in these studies was 12 min. at 24–25 in. of mercury. The treated seeds were air dried on blotting paper prior to sowing.

For the identification of physiologic races in smuts the two methods of inoculation were again used. Reference will be made to their particular use later in the text.

All mature plants were scored for number of smutted heads after ear emergence. A plant which had only one infected tiller was regarded as susceptible and marked infected.

RESULTS

The results of these experiments are presented in Tables 1 and 2. Table 1 shows percentage infection following inoculation with dry spores, while Table 2 presents the results obtained with the partial vacuum technique.

(1) *Discussion of infection following inoculation with dry spores* (*Table* 1)

From Table 1 it is seen that cultures L_3 and C_1 behaved differently; culture C_1 showing considerably greater virulence than culture L_3. Comparing the diploid samples, nos. 5, 13, 17, 19, 24, 28, 29, 34, 35, 42 and 48 (subsp. *strigosa*); samples nos. 9 and 50 (proles *brevis*); sample no. 8 (subsp. *strigosa* proles *nuda*); sample no. 12 (subsp. *Wiestii*); were all susceptible to culture C_1 and resistant to culture L_3. Samples nos. 1 and 51, both hexaploid species, were, on the other hand, susceptible to culture L_3 and resistant to culture C_1. Nine samples, all representing subsp. *strigosa*, nos. 14, 15, 16, 18, 31, 44, 45 and 52 were susceptible to both cultures of smut. Resistance to both cultures was shown by twelve diploid samples (nos. 2, 6, 11, 22, 30, 32, 33, 36, 37, 38, 39 and 53), four tetraploid samples (nos. 4, 10, 20 and 21), and two hexaploid samples (nos. 26 and 27).

TABLE 1. *Percentage of smut infection following the inoculation of de-hulled seeds with dry spores*

Species	Sample no.	Culture number					
		L_1	L_2	L_3	C_1	C_2	C_4
A. fatua subsp. *sativa* (L.) Thell	1	0	33	81	0	—	0
	26	10	0	0	0	—	0
A. strigosa subsp. *strigosa* (Schreb.) Thell	3	66	—	—	100	—	—
	5	36	0	0	100	—	0
	13	0	0	0	100	—	0
	14	0	—	50	85	—	0
	15	0	—	36	100	—	—
	16	—	—	50	100	—	—
	17	—	—	0	100	—	—
	18	50	0	25	65	—	0
	19	66	—	0	100	—	0
	24	0	0	0	66	—	0
	28	56	—	0	75	—	0
	29	32	—	0	100	—	0
	31	65	80	100	85	—	15
	34	60	22	0	60	—	—
	35	50	—	0	25	—	—
	42	—	—	0	100	—	—
	44	—	—	100	100	—	—
	45	—	—	100	100	—	—
	46	—	—	0	100	—	—
	47	—	—	50	100	—	—
	48	—	—	0	66	—	—
	49	—	—	0	50	—	—
	52	16	0	50	25	60	0
A. fatua subsp. *sativa* proles *Chinensis* Fisch.	51	—	—	75	0	—	—
A. strigosa subsp. *strigosa* proles *brevis* (Roth) Thell	9	0	0	0	50	—	0
	50	—	—	0	100	—	—
A. strigosa subsp. × proles *brevis*	25	0	0	0	100	—	0
A. strigosa subsp. *Wiestii* (Steud.) Thell	12	0	—	0	75	—	—
	23	—	—	100	—	—	—
A. strigosa proles *nuda* (L.) Hausskn.	8	0	0	0	65	—	0

Note: — indicates not tested.

The following samples were not infected by any of the smut cultures with which they were inoculated and have been omitted from Table 1. *Avena fatua* subsp. *sativa*, no. 27; *Avena strigosa* subsp. *strigosa* nos. 2, 6, 11, 30, 32, 33, 36, 37, 38, 39, 40, 41 and 53; *Avena barbata* subsp. *barbata*, nos. 4, 10 and 20; *A. strigosa* subsp. *strigosa* proles *brevis*, no. 22; *A. strigosa* subsp. *abyssinica*, no. 21.

From these results it appears that among the diploids a high degree of resistance to both cultures occurred, and likewise all the tetraploids which were adequately tested gave complete resistance. Since it is clear that L_3 and C_1 were physiologically distinct, the type of resistance appears to cover infection by more than one race.

TABLE 2. *Percentage of smut infection following inoculation of seed by spore suspension under partial vacuum*

Species	Sample no.	Culture number					
		L_1	L_2	L_3	C_1	C_2	C_4
A. strigosa subsp. *strigosa* (Schreb.) Thell	5	35	0	0	71	64	0
	6	0	0	0	1	0	0
	18	21	12	23	83	85	0
	19	37	15	29	66	72	0
	24	0	0	0	88	0	0
	28	86	0	2	92	95	0
	29	58	0	1	94	98	4
	31	98	82	76	98	92	10
	34	30	16	13	96	91	0
	52	13	0	0	50	56	0
A. strigosa subsp. *strigosa* × proles *brevis* (Roth.) Thell	25	2	0	0	96	74	0
A. fatua subsp. *sativa* (L.) Thell	27	0	3	0	0	0	0

The following varieties were not infected by any of the cultures with which they were inoculated and have been omitted from Table 2: *A. strigosa* subsp. *strigosa*, nos. 2, 11, 30 and 53; *A. strigosa* subsp. *barbata*, nos. 4, 10 and 20; *A. strigosa* subsp. *abyssinica*, no. 21; *A. strigosa* subsp. *strigosa* proles *brevis*, no. 22; *A. fatua* subsp. *sativa*, no. 26.

Unfortunately, the pathogenic range of C_1 and L_1 within the tests cannot be adequately compared because the data are incomplete. Ten samples, nos. 3, 5, 18, 19, 28, 29, 31, 34, 35 and 52 (all representing subsp. *strigosa*) were attacked by both cultures, although the degree of infection was slightly lower following inoculation by culture L_1. Clear differences between the two cultures became evident, however, in the resistance and susceptibility of several samples including sample 9 (proles *brevis*), sample 8 (subsp. *strigosa* proles *nuda*), sample 12 (subsp. *Wiestii*) and sample 25 (hybrid subsp. *strigosa* × proles *brevis*). Culture C_1 appeared to possess a wider range of infection over the material under test.

Cultures L_2 and L_3 resembled each other very closely in their pathogenicity over the range of samples. Slight differences were recorded between these two smut cultures on samples 1, 34 and 52. In view of the results presented in the next table this difference may well have been fortuitous and it may be tentatively suggested that cultures L_2 and L_3 are physiologically identical.

No definite conclusion can be drawn on the pathogenicity of culture C_2 because the tests conducted were not sufficiently extensive. Culture C_4 appears to be clearly differentiated from the rest of the cultures under study in its very narrow range of pathogenicity. Only one sample, no. 31 (subsp. *strigosa*) was infected with this culture and the degree of infection produced was very low.

(2) *Discussion of infection following inoculation by the partial vacuum method—field trials* (*Table* 2)

Nine samples were infected with culture L_1. The degree of virulence ranged from 2% in sample no. 25 (subsp. *strigosa* × *brevis*), to 98% in sample no. 31 (subsp. *strigosa*). When the pathogenicity of this culture in the field is compared with the results already described in the previous section, it will be noted that the same varieties, with only one exception, were smutted under both conditions of inoculation. The one exception, no. 25, did not become infected after inoculation with dry spores and showed only very slight susceptibility after inoculation under partial vacuum. Apart from this single sample, the results of the two tests correspond closely as regard both the range of pathogenicity, and degree of virulence.

Cultures L_2 and L_3 were very similar in their range of pathogenicity in the field, confirming the results described in the previous section. Samples nos. 18, 19, 31 and 34 (all subsp. *strigosa*) were moderately to highly susceptible to both the cultures. Traces of infection were observed with culture L_3 on samples nos. 28 and 29 (subsp. *strigosa*) but not with culture L_2, whereas some infection was noticed with culture L_2 on sample no. 27 (*Avena fatua* subsp. *sativa* (L.) Thell.) and none with culture L_3. This slight difference cannot be taken as establishing differences in the two cultures because where infection is slight, fortuitous results indicating resistance and susceptibility can easily occur. The two cultures are clearly definable from culture L_1 both in their range of pathogenicity and degree of virulence.

A high degree of similarity was also observed between the cultures C_1 and C_2 both in their pathogenic range and in their virulence, but their reaction on sample no. 24 serves to establish a physiologic difference between the two. Nine samples, all representing subsp. *strigosa*, were more or less equally susceptible to both the cultures, whereas only C_1 produced infection on sample no. 24. The high degree of infection by C_1 on this latter sample enables one to conclude with fair certainty that C_1 and C_2 are distinct races. Culture L_1 differed from culture C_1 only in that samples nos. 24 and 25 were immune or highly resistant to culture L_1 whereas they both showed susceptibility to culture C_1. C_2 differed from L_1 in that the former was effective on sample no. 25.

Culture C_4 showed very weak virulence and, also, its range of pathogenicity was remarkably narrow as compared with other cultures. This characteristic of C_4 has already been referred to in the discussion on Table 1, when this culture was tested under controlled conditions.

Further results on physiologic specialization is provided by the results of inoculation of tester varieties obtained from the U.S.A. These are presented and discussed below.

PHYSIOLOGIC SPECIALIZATION OF OAT SMUTS

Differential reactions of various varieties of oats to different smut cultures were recorded by Reed as early as 1924, when he showed that *A. strigosa* and *A. brevis* were resistant to both the smuts while most varieties of *A. sativa* were susceptible, the only exception being the variety Black Mesdag.

In an extensive investigation, Reed (1940) differentiated twenty-nine races of *Ustilago avenae* and fourteen races of *U. kolleri* on the basis of their behaviour on varieties of nine species of *Avena*. Approximately two hundred varieties and strains were used for the experiment. From these results it was concluded that seventeen varieties of oats were required for the identification of loose smut races and ten for races of covered smut. It was observed that some specialized races were sharply limited to a few varieties, while others infected a wide range of hosts belonging to several species of *Avena*. One or more varieties of all the species tested were susceptible to at least one race of either loose or covered smut.

Reed & Stanton (1942) demonstrated the susceptibility of the variety Victoria (a variety highly resistant to all the then known races of loose smut and covered smut) to a collection of loose smut isolated from the variety Fulghum.

The investigation of Tervet (1940, 1941) in which it was possible to identify only three races of each of loose and covered smut, showed that the same race possessed a wide variation in degree of infection in different years, and emphasizes the importance of ascertaining the limits of variability before conclusions are drawn on the distinctness of races. These results are in contrast to Reed's (1940) results where a high degree of constancy in the degree of reaction from year to year was obtained.

Holton & Rodenhiser (1948) also stressed the importance of determining the range of variability for accurate race identification, and, in a study over 4 years, using ten differential varieties, only fifteen races of *Ustilago avenae* and seven of *U. kolleri* were established. Varieties with 10% or more infection (on head counts) were taken as susceptible whereas those below 10% were recorded as resistant. Further work by Cherewick (1951), where the varieties Mable and Bacon were added to the ten selected by Holton & Rodenhiser (1948), showed that twenty-three races of *U. avenae* and ten of *U. kolleri* could be separated.

RACE IDENTIFICATION

Inoculated samples of the tester varieties were sown both in pots in the greenhouse and under field conditions. The inoculation method was by dry spore and spore suspension in partial vacuum respectively. A summary of the results obtained is presented in Table 3.

RESULTS

Culture L_1 when tested in the field produced a slight infection only on the variety Anthony. Unfortunately no material of L_1 was available for the pot tests so that confirmation of the field results was not possible. However, the same smut culture was highly successful on some of the experimental varieties of oats, e.g. nos. 28, 31 and a few others, so that its virulence cannot be questioned and one can conclude with a certain amount of reliability that the other varieties were resistant to this culture. The indications, therefore, are that this culture is different from any studied by Holton & Rodenhiser where none of the races was confined to Anthony. Since the testers used by Reed were different from those used in the present investigation, it is impossible to compare the reaction of L_1 with any one of his twenty-nine races of *Ustilago avenae*.

TABLE 3. *Percentage infection of tester varieties inoculated with different cultures of loose and covered smuts*

Tester variety	Field test								Pot test				
	L_1	L_2	L_3	L_4	C_1	C_2	C_3	C_4	L_2	L_3	C_1	C_2	C_4
Anthony	3	25	30	2	0	0	24	0	100	100	0	0	50
Black Diamond	0	13	14	0	0	0	0	0	50	80	0	0	0
Victory	0	15	27	0	0	0	0	0	60	100	0	0	0

The remaining tester varieties, Gothland, Monarch, Black Mesdag, Camas, Nicol, Lelina and Fulghum were not infected by any of the cultures.

Cultures L_2 and L_3 were very similar in their pathogenic range and virulence on the tester varieties. It will be recalled that the infection of the experimental samples also showed the similarities of these cultures. The varieties Anthony, Black Diamond and Victory were infected by the two cultures both in the field and in pot experiments, though some variations in their virulence was evident. On referring to Table 1 of Holton and Rodenhiser it is found that the infection capacities of the two cultures are the same as that of race A-1 of *U. avenae*, and it appears on the present evidence and using the above identifier varieties that L_2 and L_3 used in the present work are identical with race no. 1 of Holton & Rodenhiser.

Only a small amount of culture L_4 was available. In the field experiment where the percentage of infection was low it infected only one variety, Anthony. This might be due to poor viability of the spores and the very small amount of spores available. The reaction of this culture was very much like that of culture L_1, but unfortunately a comparison of the performance of these two cultures on the experimental samples is not available and therefore their relationship remains uncertain.

It has already been noted that cultures C_1 and C_2 were very similar in their reaction on the experimental samples, where a differential reaction was observed only on sample no. 24. The two cultures also resembled culture L_1 on the experimental samples but, unlike L_1 they failed to infect any of the tester varieties either in the field or pot experiments.

It will be seen, therefore, that on the basis of infection of identifier varieties C_1 and C_2 must be designated as one and the same race. When, however, the results of inoculation of the experimental sample no. 24 are considered, it becomes clear that these two races are physiologically different, and that, therefore, the ten varieties selected by Holton & Rodenhiser (1948) were insufficient for the complete identification of these races of covered smut.

The behaviour of culture C_3 was also very characteristic. As with cultures L_1 and L_4 it also infected only one of the tester varieties, i.e. Anthony. However, the percentage of infection was higher than with cultures L_1 and L_4. In the absence of any other experimental data it is very difficult to state whether this higher percentage of infection was due to greater viability of the chlamydospores or to the culture being more virulent and differing physiologically from the others. The initial culture was very small and there was insufficient material left for inoculating the tester varieties under greenhouse conditions, and further work is required to establish its identity with certainty.

The remaining culture, C_4, failed to infect any of the tester varieties in the field experiment but infection of the variety Anthony occurred in the pot experiments. The amount of inoculum available was very small, and the viability of spores was very low compared with the other cultures. It was observed in all the tests involving the experimental samples, that infection was always weak when this culture was involved and that the range of pathogenicity was very narrow.

In conclusion, it may be tentatively suggested that the eight cultures tested (four of *U. kolleri* and four of *U. avenae*) can be separated into six distinct cultures when the results of infection on experimental samples are considered in addition to those on identifier varieties. Cultures L_2 and L_3 (both *U. avenae*) represent one and the same race as far as can be judged from the present data. Culture L_1 and L_4 (both *U. avenae*) cannot be definitely separated on the basis of the results presented and the indication is that these also may be representative of one race only.

GENERAL DISCUSSION

The results of the present study, though limited mostly to the subsp. *strigosa* of *Avena strigosa*, indicate conclusively the presence of disease resistance in the lower chromosome group. For ease in discussion a summary of the reaction of samples resistant to at least one culture is given in Table 4 below.

It will be noted that samples nos. 2, 6, 11, 22, 30, 38 and 53, representing *A. strigosa* subsp. *strigosa* (diploid group); samples nos. 4, 10 and 20, representing subsp. *barbata* and sample no. 21 representing subsp. *abyssinica* (tetraploid group) were resistant to all of the smut cultures to which they were subjected. The high degree of resistance shown by the *barbata* samples in these tests contrasts sharply with Reed's (1940) findings in which this species proved to be completely susceptible to both smuts.

The production of resistant, amphidiploid hybrids for gene transference, and the use of this technique in crop improvement requires the thorough investigation of possible parental species both for the existence of resistant genes as well as for their range of polymorphism in respect of such genes. The data presented above shows clearly the importance of fully appreciating the degree of variability in these self-fertilizing species and subspecies. In a total of thirty-seven samples of subsp. *strigosa* of *Avena strigosa*, for example, only six possessed resistance to all the smut cultures tested. The rest were susceptible to one or more of the physiologic forms with which they were inoculated. It becomes clear, therefore, that one cannot take a representative specimen of any one species and assume it to be resistant or susceptible. Material to be used in hybridization work should be tested beforehand and on individual and not on a specific basis. The indiscriminate use of parental

TABLE 4. *Summary of the reaction of experimental samples to the smut races investigated*

(Only samples showing some resistance have been included in this table)

Species	Sample no.	Smut				
		L_1	L_2 and L_3	C_1	C_2	C_4
A. strigosa subsp. *strigosa* (Schreb.) Thell.	2	R	R	R	R	R
	3	S	—	S	—	—
	5	S	R	S	S	R
	6	R	R	R	R	R
	11	R	R	R	R	R
	18	S	S	S	S	R
	19	S	S	S	S	R
	24	R	R	S	R	R
	28	S	R	S	S	R
	29	S	R	S	S	S
	30	R	R	R	R	R
	32	—	R	R	—	—
	33	—	R	R	—	—
	34	S	S	S	S	R
	36	—	R	R	—	—
	37	—	R	R	—	—
	38	R	R	R	—	R
	39	—	R	R	—	—
	42	—	R	S	—	—
	46	—	R	S	—	—
	49	—	R	S	—	—
	52	S	R	S	S	R
	53	R	R	R	R	R
A. strigosa subsp. *barbata* (Pott.) Thell	4	R	R	R	R	R
	10	R	R	R	R	R
	20	R	R	R	R	R
A. strigosa subsp. *strigosa* proles *brevis* (Roth.) Thell	9	R	R	S	—	—
	22	R	R	R	R	R
	50	—	R	S	—	—
A. strigosa subsp. *strigosa* × proles *brevis*	25	R	R	S	S	R
A. strigosa subsp. *abyssinica* (Hochst.) Thell	21	R	R	R	R	R
A. fatua subsp. *sativa* (L.) Thell	26	R	R	R	R	R
	27	R	R	R	R	R

Note: R = Resistant and S = Susceptible.

material from a species in hybridization, without precise knowledge of the individual, can lead to quite undesirable end results.

The above remarks on intra-specific differences apply equally to intra-varietal variation. Character variations are frequently observed within oat varieties and the results presented above show that morphologically identical individuals (e.g. nos. 28 and 34) belonging to subsp. *strigosa* possessed a different pattern of resistance to the races under test. This underlines the importance of making a genotypic analysis of the plants before use is made of them in the production of new hybrids for breeding. Furthermore, the existence of non-morphological variations within varieties may easily lead to the selection of lines within a single variety which behave differently to the same race of the pathogen, thus giving quite an erroneous picture of racial specialization. Brown (1937) emphasized this point and showed that individual plants of each of the two differential varieties, Ruakura and Green Mountain, behaved differently to a pure culture of *Puccinia coronata*.

Adequate precautions must therefore be taken if a satisfactory world list of physiologic races is to be compiled, and periodic testing of the differential hosts appears to be essential in order to check on the appearance of mutant and recombinant forms, possessing altered reactions to resistance. Furthermore, the differentials should, for the above reasons, be thoroughly pure-lined before use and in order to obtain uniformity such work should be centralized. With the development of the bulk method of breeding, self-fertilizing crop varieties are nowadays put on the market in a lower state of purity than was otherwise the case, and the use of such varieties as differentials would render work on racial identification practically valueless.

Although it was found in the present study that environmental conditions affected the virulence and range of pathogenicity of the fungal cultures to only a slight degree, no discussion on race identification studies would be complete without reference to the effect of environmental factors. Various effects of the environment have been demonstrated by many workers. Rodenhiser & Holton (1942) showed in *Tilletia laevis* and *T. tritici* that a host variety when inoculated with a pure culture and sown at two different experimental stations behaved differently. At one location the variety was highly to moderately resistant, whereas in the same year at the other centre, it was highly susceptible. The present field experiments also indicate that differences in smut infection results can be obtained in different years due to fluctuation in the environment.

Further, one must consider the importance of variation and recombination within single, hetero-karyotic races. In *Ustilago*, infection takes place by means of dikaryotic mycelium. Such dikaryophytes may or may not be heterokaryotic for infection type and infection range. Recombination through the independent segregation of mating type and infectivity may give rise to entirely new recombinations showing altered host specificity. The use of paired monosporidial cultures s/ ha~~ve~~ been advocated by Sampson & Western (1938) as the only certain method of reducing this source of variability in racial identification studies, on the smuts. It will be realized that such mating of pairs of spores from the basidium will have to be carried out for at least two generations before certain purity is achieved.

Difficulties were encountered in the present studies in identification of the races owing to an inadequate number of tester varieties. Hassebrauk, quoted by Reed (1946), working on *Puccinia triticina* found that the differential hosts appropriate for one country may not be suitable for another. Further Rashevskaya & Barmenkoff (see Reed, 1946), found the inadequacy of the established tester varieties for the identification of physiologic races of leaf rust of wheat in Russia, and Honeker (see Reed, 1946) reported that the varieties of barley used by Mains and Dietz were unsatisfactory for differentiation of barley mildew races in Germany. Likewise in the present study, the smut differentials selected by Holton & Rodenhiser (1948) were insufficient for the exhaustive identification of smut races in this country. It seems, therefore, that the present list of testers will have to be considerably extended to enable a compilation to be made of physiologic forms in the important species of plant pathogens.

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DRY-ROT DISEASE OF THE POTATO

VIII. A STUDY OF THE PATHOGENICITY OF *FUSARIUM CAERULEUM* (LIB.) SACC. AND *FUSARIUM AVENACEUM* (FR.) SACC.

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(With 5 Text-figures)

Inoculation of wounded tubers at intervals throughout the storage season showed that, after harvesting, resistance to infection by *F. avenaceum* was maintained for a longer period than resistance to *F. caeruleum*, although eventually the tubers became equally susceptible to both fungi. Tubers were less readily infected through clean-cut than through scarified wounds. Resistance to infection was greater when an interval elapsed between wounding and inoculation than when the wound was inoculated immediately; this was related to an increase in the intrinsic ability of the cells near the wound to resist infection rather than to suberization of the wounded surface or wound periderm formation.

Histological studies showed that *F. caeruleum* grew through the intercellular spaces and that the adjacent host cells remained alive, often for considerable periods, whereas *F. avenaceum* killed and penetrated the cells with which it came into contact. Restriction of incipient or established lesions caused by *F. caeruleum* and of incipient lesions caused by *F. avenaceum* was associated with suberin deposition on the host cell walls and in the intercellular spaces; in established lesions caused by *F. avenaceum*, restriction was attributed to an increase in the intrinsic resistance of the adjacent host cells, similar to that found near a wounded surface.

The two fungi most frequently associated with dry-rot disease of the potato tuber in Britain, *Fusarium caeruleum* (Lib.) Sacc. and *F. avenaceum* (Fr.) Sacc., are widely distributed in field soils in seed-producing areas in Scotland (unpublished work) and are probably equally common in other parts of the country, judging from the diverse sources of samples of diseased tubers from which *F. caeruleum* has been isolated and from the widespread occurrence of cereal foot rot due to *F. avenaceum*. Under laboratory conditions both fungi cause active tuber rots (Moore, 1945). Isolations from more than 140 samples of diseased tubers from commercial stocks of seed over a period of 3 years showed, however, that *F. caeruleum* was responsible for 93 % of roots attributable to parasitic Fusaria, while *F. avenaceum* was responsible for only 6 % (McKee, 1952). A similar wide difference in the commercial importance of these two fungi as causal agents of dry rot has been reported from the U.S.A. (Sherbakoff, 1915), Germany (Schmidt, 1928), France (Lansade, 1950), and Holland (Mooi, 1950). The present investigation was undertaken to ascertain the reasons for this difference.

The temperature and relative humidity necessary for maximum spread of rot were shown by Moore (1945) to be higher for *F. avenaceum* than for *F. caeruleum*. Lesions caused by *F. avenaceum* were found by Schmidt (1928) to be permanently restricted* by temporary exposure to conditions unfavourable to the growth of the fungus, while lesions caused by *F. caeruleum* spread again when conditions became more favourable. The disparity in the relative importance of the two fungi as tuber parasites suggested, however, that further differences in behaviour must exist between them; various other aspects of their pathogenicity have, therefore, been studied. Particular attention has been paid to ability to initiate infection in relation to the variation of tuber susceptibility with maturity (time in storage), a factor shown by Lansade (1950) and Boyd (1952*b*) to be of importance with regard to *F. caeruleum*. The relationship of the process of wound healing to resistance and the histology of the lesions have also been investigated.

MATERIALS AND METHODS

The varieties Doon Star and King Edward were used because of their susceptibility and relative resistance, respectively, to dry rot. Samples of tubers for any particular series of experiments were taken from the same stocks, stored either in sacks (var. Doon Star) or in bulk (var. King Edward) in frost-proof buildings, and were washed and surface sterilized before inoculation.

Isolates of *F. caeruleum* and *F. avenaceum*, obtained originally from diseased tubers, were maintained on sterilized soil. The 'wild type' forms of both fungi were retained throughout the investigation, as shown by periodic subculturing on potato dextrose agar. *F. caeruleum* spored copiously when plates of potato dextrose agar were seeded with small quantities of soil from the stock cultures. *F. avenaceum*, on the other hand, spored irregularly on agar media but produced considerable numbers of spores in small groups scattered throughout soil cultures. For inoculation, spore suspensions of the two fungi were prepared, from the agar plates and the soil cultures respectively, by suspending portions of the cultures in water and diluting to the required level.

In infection studies the tubers were wounded by removing a slice of tissue lengthwise, to a maximum depth of about 0·5 cm., with a sharp knife; the exposed tissues thus consisted of storage parenchyma. In some cases, where stated, more severe wounds were inflicted by scratching the plain wounded surface with the point of the knife; the scarified area was about 1·5 cm.sq. A drop of spore suspension (about 120,000 spores/ml.) was put on the centre of each wound, using a dropper giving 75 drops/ml. Germination of the spore suspension was checked in all cases and was found to range from 50 to 100 %. The inoculated tubers were kept at room temperature (*c.* 16–20° C.), in containers lined with wet blotting paper, for 4 weeks. The tubers were then cut and the appearance and size of the lesions recorded.

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* Lesions are called 'restricted' when their extension into adjacent host tissue is hindered or prevented for any reason; causes of restriction are described on p. 430.

Representative lesions, with adjacent healthy tissue, were sectioned, using a freezing microtome. The extent of suberization in tubers inoculated some time after wounding was also examined in a tuber selected at random from those due for inoculation.

RESULTS

Types of lesions

(*a*) *Lesions caused by* F. caeruleum

A brown speckling of the tissues immediately beneath the inoculated surface normally developed within 3–7 days. This initial speckling appeared more rapidly and was more marked in tubers resistant to infection in which the small spots did not develop further; in susceptible tubers the spots increased in size and coalesced, and the rot soon extended into the adjacent tissues. All gradations between these extremes were noted in tubers where the spread of the fungus had been arrested but restriction of lesions occurred more commonly in the earlier stages. Restricted lesions could be identified both by their size and by the appearance of the margin which was more sharply defined than that of the spreading lesions. On cutting, the appearance of the lesions was similar to that in natural infections. The tissues in lesions formed early in the storage season remained firm and coherent but in tubers inoculated at later periods the diseased areas sometimes became soft and mushy.

The dimensions of lesions in susceptible tubers indicated that the rate of spread of rot was similar in all directions. In more resistant tubers the rot tended initially to spread faster from the inoculated area towards the interior of the tuber, before extending laterally in the tissues further away from the wounded surface.

(*b*) *Lesions caused by* F. avenaceum

After successful inoculations, the infected tissues blackened and collapsed rapidly, giving rise to pits in the surface. Necrosis was visible within 3 days in susceptible tubers in which typical spreading lesions later developed. In resistant tubers, there was rarely any indication that penetration had occurred, even speckling of the inoculated surface being infrequent.

The shape of the rotted area indicated that initially *F. avenaceum* spread more freely inwards from the point of inoculation than laterally; this effect was more marked in more resistant tubers. Lateral spread was often restricted in tuber samples in which *F. caeruleum* spread evenly in all directions.

As in *F. caeruleum* infections, restriction occurred in lesions of all sizes, though more commonly in the earlier stages of infection. Restricted lesions of larger size showed a zone of translucent tissue (about 0·5 cm. in width) between the discoloured tissues of the lesions and the unaltered flesh.

Factors influencing the susceptibility of tubers to infection by F. caeruleum *and* F. avenaceum

(*a*) *Maturity of tubers*

Samples of tubers were inoculated at intervals throughout the 1951–52 storage season, to determine whether susceptibility to *F. avenaceum* and to *F. caeruleum* changed concurrently. The lesions were classified as spreading or restricted—the latter showed some spread beyond the initial speckling but were not growing actively at the time of the final examination. The results are summarized in Table 1.

Table 1. *Percentage infection resulting from inoculation at intervals throughout the storage season* 1951–52 (*one inoculation per tuber*)

	F. caeruleum			*F. avenaceum*		
Date	No. of tubers inoculated	Tubers with spreading lesions (%)	Tubers with restricted lesions (%)	No. of tubers inoculated	Tubers with spreading lesions (%)	Tubers with restricted lesions (%)
			Doon Star			
8. xi. 51	5	0*	—	—	—	—
21. xi. 51	10	20*	—	—	—	—
5. xii. 51	20	55	0	20	0	5
2. i. 52	20	30	25	20	5	5
4. ii. 52	20	85	15	20	5	25
4. iii. 52	5	100	0	10	60	10
21. v. 52	5	80	20	10	100	0
			King Edward			
20. xii. 51	20	0	0	20	0	0
22. i. 52	20	0	10	20	0	15
20. ii. 52	20	20	10	20	0	25
7. iv. 52	20	45	30	20	35	10
23. vi. 52	20	100	0	20	100	0

* Lesions not subdivided into spreading and restricted.

In both varieties, resistance to infection decreased as the storage season advanced, but the variety Doon Star lost its resistance more rapidly than King Edward. Resistance to infection by *F. avenaceum* was maintained for a longer period than resistance to *F. caeruleum*.

During the 1952–53 season, inoculation of Doon Star tubers with spores of the same isolate of *F. caeruleum* showed that the decrease in resistance occurred earlier than in 1951–52 (Table 2).

Isolated experiments indicated that loss of resistance in tubers of the variety King Edward also occurred earlier in the 1952–53 season than in the 1951–52 season; thus 80% of the tubers inoculated with *F. caeruleum* on 14. i. 53 developed spreading lesions, the corresponding figure in the 1951–52 season being 0%.

(*b*) *Type of wound*

The results of inoculation of sacrified wounds are given in Table 3. These are directly comparable with the results of inoculation of plain wounds given in Table 1.

TABLE. 2. *Comparison of results of periodic inoculation of tubers of the variety Doon Star with* F. caeruleum *during the* 1951–52 *and the* 1952–53 *seasons*

Date	Tubers with spreading lesions (%)	Date	Tubers with spreading lesions (%)
8. xi. 51	0	31. x. 52	80
5. xii. 51	55	3. xii. 52	100
2. i. 52	30	13. i. 53	100
4. ii. 52	85	10. ii. 53	100

TABLE 3. *Percentage infection resulting from inoculation of scarified wounds on tubers, at intervals throughout the storage season* 1951–52 (*one inoculation per tuber*)

	F. caeruleum			*F. avenaceum*		
Date	No. of tubers inoculated	Tubers with spreading lesions (%)	Tubers with restricted lesions (%)	No. of tubers inoculated	Tubers with spreading lesions (%)	Tubers with restricted lesions (%)
		Doon Star				
21. xi. 51	10	40*	—	—	—	—
5. xii. 51	20	60	0	20	35	25
2. i. 52	20	60	5	20	45	55
4. ii. 52	20	85	15	20	45	45
3. iii. 52	2	100	0	10	100	0
21. v. 52	5	100	0	5	100	0
		King Edward				
20. xii. 51	20	5	45	20	10	25
22. i. 52	20	30	70	20	55	45
20. ii. 52	20	65	30	20	30	40
7. iv. 52	20	80	10	25	100	0
23. vi. 52	19	100	0	20	100	0

* Lesions not subdivided into spreading and restricted.

In tubers of the variety Doon Star inoculated with *F. caeruleum*, infection of the scarified wounds was, in general, only slightly greater than that of the plain wounds at the same date. Tubers of the variety King Edward inoculated with the same fungus, however, developed considerably more infection from the scarified wounds, as did those of both varieties inoculated with *F. avenaceum*. Resistance to infection diminished with increasing maturity of the tubers and was maintained longer against *F. avenaceum* than *F. caeruleum* in the variety Doon Star.

(*c*) *Interval between wounding and inoculation*

Samples of either five or ten tubers, kept under favourable conditions for suberization, were inoculated at intervals after wounding. When inoculation was delayed, the drop of spore suspension spread much less than on the fresh wounds, giving a higher concentration of spores at the site of inoculation. The results of these inoculations are shown in Fig. 1; only the spreading lesions are included as the data for the restricted lesions followed a similar pattern. The extent of suberization was determined in each case (Table 4).

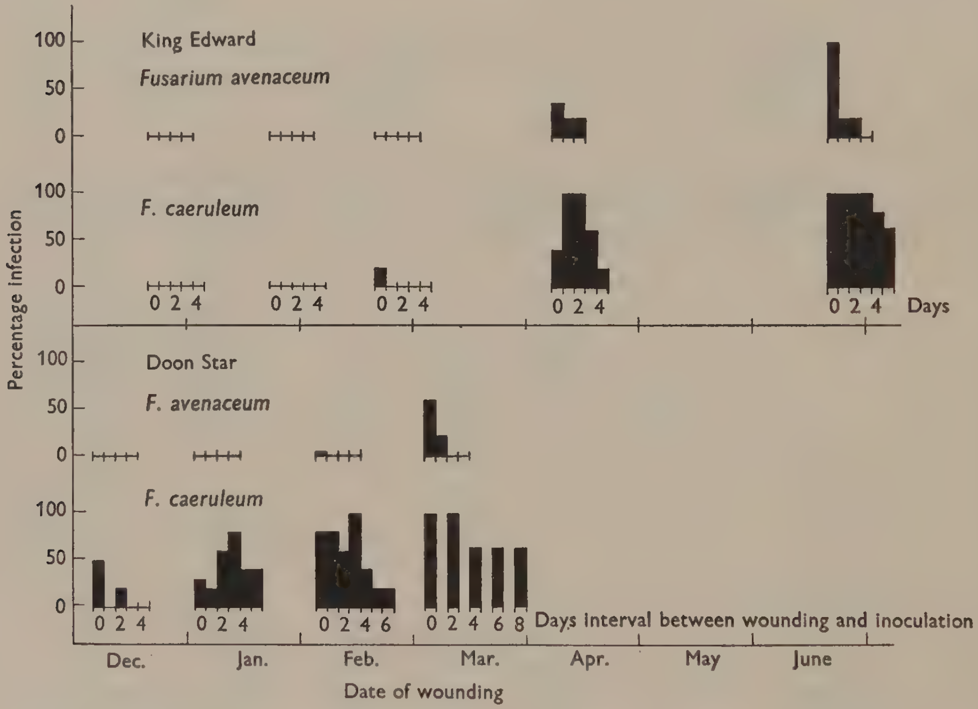

Fig. 1. Infection resulting from inoculation of tubers of the varieties Kind Edward and Doon Star at intervals after wounding. Date of wounding in each group indicated by position of initial inoculation (0 days) in relation to monthly scale.

The variety Doon Star remained susceptible to infection by *F. caeruleum* for a considerable time after wounding, the more mature the tuber the greater being the period of susceptibility. The variety King Edward, on the other hand, showed little infection at all by *F. caeruleum* early in the season, but later in the season the tubers remained susceptible for some days after wounding. Tubers of both varieties showed little infection by *F. avenaceum* in the earlier part of the season; later on, both remained susceptible for a time after wounding.

Although, as has been stated, *F. caeruleum* could infect tubers of the variety Doon Star for a considerable time after wounding, the lesions resulting from delayed inoculations were usually much smaller than those resulting from inoculations made at the time of wounding, even allowing for their shorter period of development on any particular date. The restriction in the growth of such lesions became more evident the greater the interval between wounding and inoculation. Lateral spread from the point of infection was also restricted in delayed inoculations in samples where the rot spread freely in all directions as a result of inoculation of freshly made wounds.

TABLE 4. *Rate of healing in wounded tubers*

Date	Age of wound in days											
	¼	½	¾	1	1½	2	3	4	5	6	7	8
					Doon Star							
28. x. 51	1	2	2	5	4	—	—	—	—	—	—	—
5. xii. 51	—	—	2	—	—	3	4	—	5*b*	—	—	—
2. i. 52	—	—	—	2	—	3	4*a*	—	5*b*	6*b*	—	—
4. ii. 52	—	—	—	2	—	3	3	3*a*	4*b*	—	5*c*	—
4. iii. 52	—	—	—	1	—	3	4	5*b*	—	5*b*	—	6*c*
21. v. 52	—	—	—	1	—	3	—	—	4*a*	—	5*b*	—
					King Edward							
20. xii. 51	—	—	2	—	—	3	—	4*a*	—	—	—	—
22. i. 52	—	—	—	1	—	3	3*a*	5*b*	—	—	—	—
20. ii. 52	—	—	—	1	—	2	3	4*b*	—	—	—	—
7. iv. 52	—	—	—	1	—	2	3*a*	—	—	—	—	—
23. vi. 52	—	—	—	1	—	2	3	4	4*b*	—	—	—

The extent of suberization at the time of inoculation was examined, sections being stained with ammoniacal gentian violet (Artschwager, 1927). The development of the suberized layer and of the cork meristem agreed with the description of Priestley & Woffenden (1923). For ease of tabulation the extent of wound healing was divided into arbitrary stages which are defined below:

Degree of suberization

0 No suberization.
1 Slight suberization of outer walls of exposed cortical cells only.
2 Suberization of outer walls of all exposed cells.
3 Suberization extending to some radial walls of exposed cells.
4 Suberization extending to all radial walls of exposed cells.
5 Suberization extending to all walls of exposed cells.
6 Suberization extending to all walls of exposed cells and to next cell layer.

Degree of meristematic activity

a Cell division in isolated cells.
b Cell division throughout—starting in nearly all cells.
c Cell division throughout—marked in all cells.

Suberization and meristem development in the samples of tubers used in the inoculation experiments recorded in Fig. 1 are given in Table 4, using the above codes.

Early in the storage season the rate of suberization of wounds appeared to be very rapid in both varieties, but it soon dropped to a lower rate which was then maintained for a considerable period. The rate of development of a meristem remained more or less constant during the period recorded.

Wounds in tubers of the variety Doon Star appeared to suberize somewhat more quickly than those in tubers of the variety King Edward, but the difference was very slight. There was no difference in the meristematic activity of the two varieties.

During the period when rate of suberization of wounds and of meristem formation remained nearly constant, the tubers became progressively more susceptible to infection by both *F. caeruleum* and *F. avenaceum*, indicating that these aspects of wound healing were not the main factors in determining resistance.

(*d*) *Response to wound stimulus*

The restriction of the spread of lesions resulting from delayed inoculations and of lateral spread of lesions in resistant tubers suggested that the cells in the vicinity of the wounded surface might have developed some resistance to infection. Such an effect was noted in a number of experiments which indicated also that the resistance developed was more effective against *F. avenaceum* than against *F. caeruleum*. In a typical experiment, ten tubers of the variety Doon Star were wounded; five were inoculated directly with a drop of *F. avenaceum* spore suspension; the other five were re-wounded after 3 days by removing a thin slice of tissue from the previously cut surface and were then inoculated as before. Two spreading and three restricted lesions developed from the first inoculations, while no indication of infection developed from the delayed inoculations, despite the re-wounding. A similar experiment, using tubers of the variety King Edward and *F. caeruleum*, gave similar results but the contrast was less marked. In another experiment tubers of the variety King Edward were cut and kept under humid conditions for 9 days, together with unwounded tubers. The latter were then wounded and the tubers originally wounded were rewounded by removing a thin slice of tissue. All the wounds were then inoculated with *F. caeruleum* spores in the usual way; the results of this experiment are given in Table 5.

TABLE. 5. *Comparison of infection by* F. caeruleum *resulting from inoculation of wounds made in the vicinity of a healed wound and in normal tissues*

Type of wound	No. of inoculations	No. of lesions	
		Spreading	Restricted
Plain, fresh wound	5	3	2
Plain, re-wounded old wound	5	1	1
Scarified, fresh wound	5	5	0
Scarified, re-wounded old wound	5	1	3

The influence of the wound-healing process on spore germination was determined by re-wounding a small area of a healed wound and applying a drop of spore suspension. Percentage germination after 18 hr. was not reduced in comparison with spores placed on a fresh wound (in a corresponding position on the opposite side of the same tuber) but the growth of the germ-tubes was restricted—the degree of restriction being greater in *F. avenaceum* than in *F. caeruleum*. Browning of the cut surface developed more rapidly in the re-wounded than in freshly wounded tissues in all these experiments.

Factors influencing the rate of spread of lesions caused by F. caeruleum *and* F. avenaceum

(*a*) *Maturity of tubers*

Lesions resulting from periodic inoculations throughout the storage season were cut after 4 week's development and the depth of the penetration measured. The average depths of rot are given in Table 6; only the lesions which appeared to be spreading when measured were included. The environmental conditions were not identical for the trials started on different dates, as the tubers were stored at room temperature (*c.* 16–18° C.); however, the temperature fluctuations were not great and it is thought that the results may be compared without undue risk of drawing false conclusions.

TABLE 6. *Average depth (in cm.) of lesions resulting from inoculations on different dates (after 4 weeks' development)*

	F. caeruleum		*F. avenaceum*	
Date	Plain wound	Scarified wound	Plain wound	Scarified wound
	Doon Star			
2. i. 52	1·5	1·7	1·0	1·7
4. ii. 52	1·5	2·1	2·0	2·0
3. iii. 52	2·0	2·0	2·3	2·4
	King Edward			
20. xii. 51	—	1·0	—	1·0
22. i. 52	—	1·7	—	1·2
20. ii. 52	1·0	1·4	—	1·5
7. iv. 52	1·6	1·6	1·9	2·3

The results given in Table 6 indicate that the tubers become less resistant to the spread of both *F. caeruleum* and *F. avenaceum* as they matured. Lesions developing from scarified wounds were larger than those from plain wounds but the proportionate increase in size of lesions is much less than the increase in percentage infection over the same period (Tables 1 and 3).

(*b*) *Temperature*

The effect of temperature on the rate of spread of rot was investigated in three varieties of potato. The tubers, after inoculation with 0·1 g. of a soil culture of the required fungus, were kept under humid conditions at 16° C. for 2 weeks to ensure infection. The size of each lesion was then measured externally in two (marked) directions at right angles. The tubers were placed in incubators at six different temperatures for a week, when the lesions were re-measured. The results of two trials, started on 22. ii. 51 and on 1. v. 51 respectively, were in good agreement; the data in Table 7 were obtained from the latter, where the rates of extension were somewhat greater than in the earlier trial, probably because the tubers had matured further. Similar results were obtained when the experiment was repeated in January 1952, using twenty tubers, stored in open trays, in each treatment but only three different temperatures—a controlled room at 21° C., a room averaging 17° C., and a store averaging 4·5° C.

TABLE 7. *Increase in external diameter of dry-rot lesions in mm. per week.* (*Mean value from eight tubers*)

		Temperature					
Pathogen	Variety	30° C.	25° C.	20° C.	15° C.	10° C.	5° C.
F. caeruleum	Doon Star	0±0·2	10±0·7	15±0·4	14±0·3	8±0·3	2±0·5
	Arran Pilot	0±0·5	8±0·6	15±0·4	12±0·5	5±0·6	1±0·5
	King Edward	0±0·1	0±0·1	9±1·5	5±1·3	1±0·4	0±0·3
F. avenaceum	Doon Star	6±0·5	15±1·1	17±0·8	14±0·6	9±0·1	4±0·4
	Arran Pilot	4±0·4	16±0·8	15±0·9	13±1·4	7±0·6	3±0·5
	King Edward	5±0·4	14±0·9	10±0·5	9±0·5	4±0·6	2±0·3

The optimum temperature for the extension of lesions caused by *F. caeruleum* lay between 15° and 20° C., while the corresponding figure for *F. avenaceum* was between 20° and 25° C. Lesions caused by *F. avenaceum* spread more rapidly than those caused by *F. caeruleum*, even at low temperatures, despite the fact that the optimum temperature was higher for *F. avenaceum*. These results agree in general with those obtained by Moore (1945), who found, however, that at 15° C. or lower *F. caeruleum* spread more rapidly than *F. avenaceum*.

Histology of lesions

A selection of the lesions which developed in the inoculation experiments were sectioned at a thickness of 100μ and were examined after staining with Thionin and Orange G (Stoughton, 1930). This method proved useful, as noted by Rudd Jones & Dowson (1950), on account of the differential staining of diseased and healthy host tissues as well as the fungus mycelium. The details of the histology of the infected tissues were found to depend on the pathogen, on the resistance of the tuber, and on the particular part of the lesion examined, and are considered under separate headings.

(*a*) *Region near site of infection by* F. caeruleum (Fig. 2)

The mycelium had penetrated intercellularly without evoking any marked response until reaching the wound meristem, where the host cells had collapsed and where hyphae were sometimes present in the dead cells. Beneath the meristem, necrotic cells with dark-staining granular contents occurred singly or in groups and were surrounded by cells whose contents stained normally but whose walls were thickened by a blue-staining 'suberized deposit' (Hill, 1939). This deposit stained red with Sudan IV and blue with ammoniacal gentian violet and appeared to be identical with the substance deposited initially on or near a wounded surface but not with the suberin of the wound periderm.

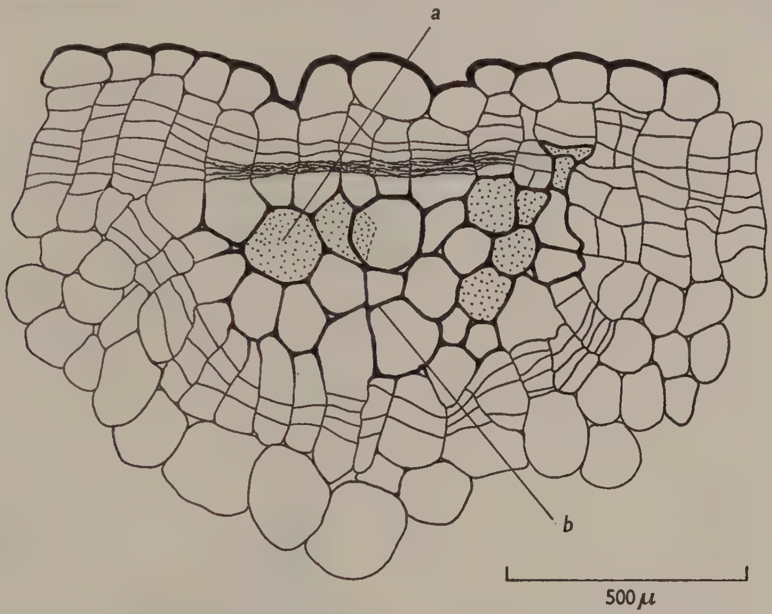

Fig. 2. Small lesion caused by *F. caeruleum*. *a*, necrotic cells; *b*, cells with walls thickened by suberin deposition.

(*b*) *Region near site of infection by* F. avenaceum

The histology was similar to that described for *F. caeruleum* but in general mycelium was more abundant, more cells had been penetrated, and necrotic cells were more frequent.

(*c*) *Spreading lesions caused by* F. caeruleum

At the advancing edge of the rot, hyphae grew sparsely between the host cells which showed little reaction to the presence of the fungus (Fig. 3). Further back in the lesion less starch was present and suberin deposition often occurred on the host cell walls and in the intercellular spaces, the extent of deposition in any particular lesion being related to the resistance of the tuber to infection; the mycelium was confined to the intercellular spaces but was usually abundant (Fig. 4). In many cases, especially where the tubers showed some resistance to infection, the host cells appeared to remain alive for a considerable period in contact with the mycelium, as indeed might be surmised from their ability to deposit suberin. The cytoplasm of such cells stained in the same way as that of normal cells, and the protoplasts could be plasmolysed. A zone in which the host cells had been penetrated occurred round the site of infection in large lesions; in the most susceptible tubers this zone

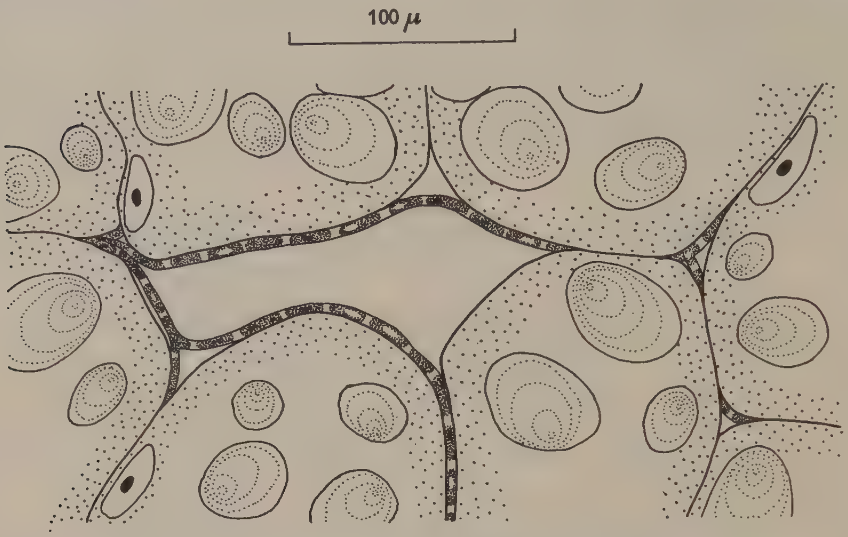

Fig. 3. Lesion caused by *F. caeruleum*—near advancing edge.

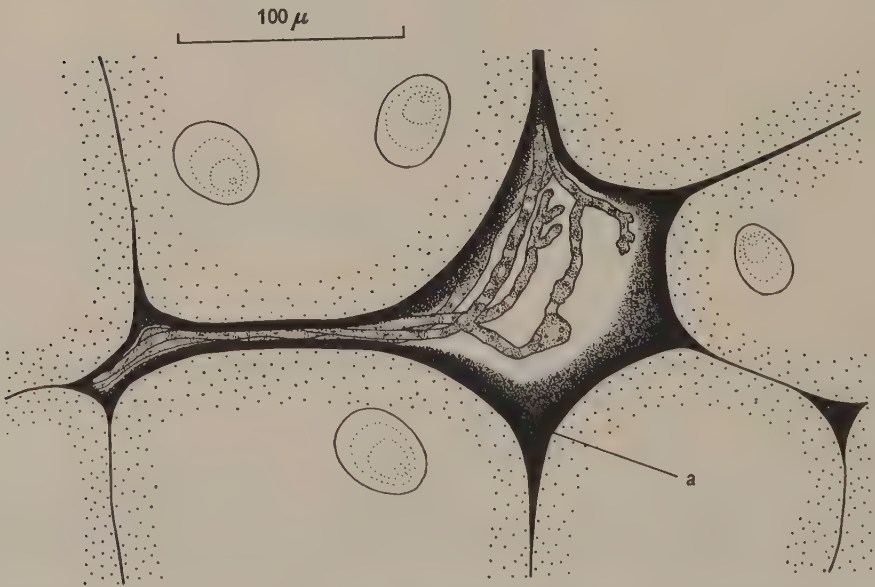

Fig. 4. Lesion caused by *F. caeruleum*—some distance behind advancing edge. *a*, suberin deposit.

extended nearly as far as the advancing edge of the lesion. Starch hydrolysis and suberin deposition did not occur in the affected tissues in very susceptible tubers, nor were the dark-staining granules, noted in cells killed in more resistant tissues, present; it would appear that such tissues had lost the ability to react to injury.

The cytoplasm of cells in the cortical region of the tuber and of cells near healed wounds showed a reaction, as indicated by pink staining with Thionin, as soon as the advancing mycelium came in contact. Subsequent suberin deposition was also very active, suggesting that these cells were more sensitive than those in the deeper layers of the tuber. Vascular tissues were sometimes more resistant than the surrounding parenchyma.

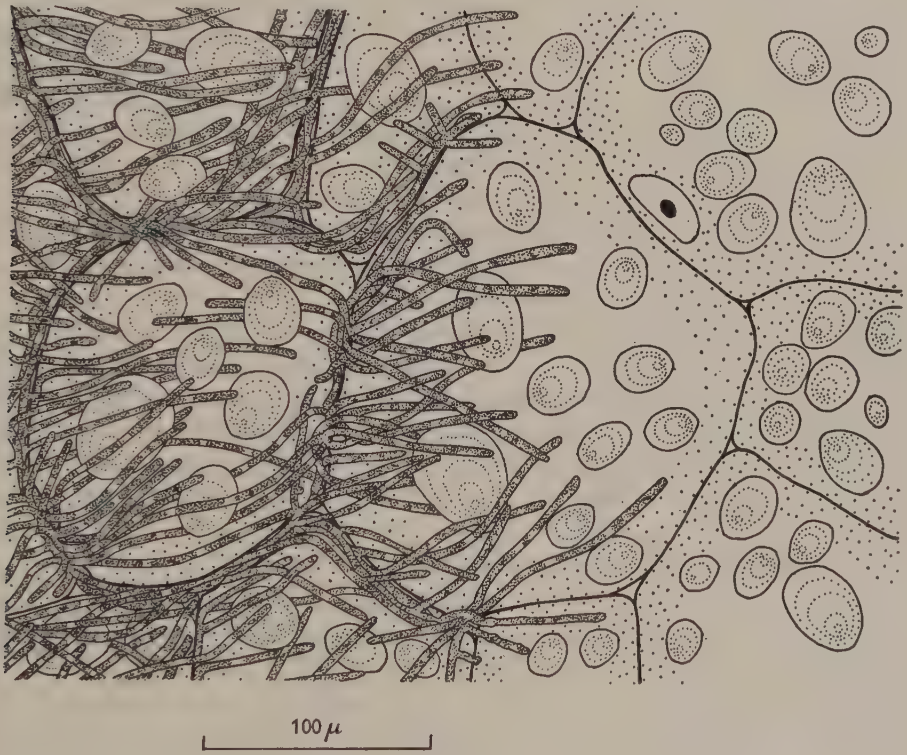

Fig. 5. Lesion caused by *F. avenaceum*—advancing edge.

(*d*) *Spreading lesions caused by* F. avenaceum

Hyphae penetrated the host cells even at the advancing edge of the lesion, where the mycelium was often abundant. The transition from healthy to diseased tissue was sharp, normal cells being separated by only one or two cells with pink-staining cytoplasm from cells packed with fungus mycelium (Fig. 5). The invaded cells were killed but did not form the dark-staining granules found near the site of infection; the starch was not hydrolysed and suberin rarely deposited.

Zones where the growth of the fungus was largely restricted to the intercellular spaces were present even in lesions in susceptible tubers, alternating with the zones of free spread. Suberin deposition was noted occasionally in such zones, sometimes in advance of the mycelium.

(*e*) *Restricted lesions caused by* F. caeruleum

Restrictions of further spread occurred at times in lesions of all sizes but was found most frequently in small lesions near the site of infection, i.e. near the wounded surface. In many cases infection did not proceed beyond the initial stages described in section (*a*) (Fig. 2). The infected region was separated from the healthy tissue by a continuous layer of cells with suberized walls. The suberized layer was sometimes penetrated, more frequently in the deeper parts of small lesions than near the wound surface where the host cells which had reacted to the wound stimulus showed marked suberin deposition. A meristem was often found between the cells with suberized deposits and the healthy tissue in the small lesions. Few starch grains were present in the healthy cells near the lesion, which developed a blue colour when treated with ferric chloride solution, a reaction stated by Johnston & Schaal (1952) to indicate the presence of chlorogenic acid; small groups of such cells often remained uninfected when surrounded by infected tissues resulting from a breakthrough in another part of the inoculated surface.

Examination of lesions which had advanced some way into the tuber before restriction, showed that suberin had been deposited in advance of the mycelium, giving a more sharply defined margin than in the spreading lesions. No meristem was laid down between the healthy and diseased tissues. Restriction of larger lesions occurred more frequently in King Edward tubers, which, in general, showed more active suberin deposition following infection than did Doon Star tubers.

(*f*) *Restricted lesions caused by* F. avenaceum

Lesions restricted near the site of infection were similar to those described for *F. caeruleum*. Larger restricted lesions were surrounded by a zone of starch-free cells. Mycelial growth was either retarded or inhibited in this zone and if present was entirely intercellular. Suberization of the cell walls was sometimes found at the junction of the starch-free and normal tissues but was only slight in amount.

DISCUSSION

F. caeruleum would appear to possess greater ability than *F. avenaceum* to initiate infection in wounded tubers. Thus *F. caeruleum* was able to cause infection earlier in the storage season than could *F. avenaceum*; it could infect a clean wound in tubers where *F. avenaceum* required a scarified wound and it could cause infection for a longer period after wounding than could *F. avenaceum*. Differences between the rates at which lesions caused by the two fungi spread through the tubers when infection had occurred were also found, but the contrast was not as great as between their invasive powers. Natural infection of seed tubers occurs mainly as a consequence of the wounding inflicted by riddling (Foister, Wilson & Boyd, 1952); this operation is usually performed at a time when the tubers are more or less resistant to infection by *F. avenaceum* but are showing varying degrees of susceptibility to *F. caeruleum*—at least for the two varieties investigated. In view of these facts, it is not surprising that by far the greater part of dry rot losses under commercial conditions was found to be caused by *F. caeruleum* (McKee, 1952). Resistance to infection by *F. caeruleum* was maintained for a longer period by tubers of the variety King Edward, which in practice is relatively resistant to the disease, than by the susceptible variety Doon Star. This is in general agreement with the findings of Boyd (1952*b*).

The difference in the host-parasite relationships in the two diseases may be related to the relative 'efficiencies' of the two fungi as tuber parasites. In spreading lesions caused by *F. caeruleum*, the hyphae grew in the intercellular spaces without causing a marked reaction in the adjacent host cells; these remained alive for some time, suggesting that a state of true parasitism was established. The hyphae of *F. avenaceum*, on the other hand, killed and penetrated the cells with which they came in contact; they developed saprophytically on the dead tissues and, in susceptible tubers, advanced by killing and colonizing the tissues at the periphery of the lesion. The behaviour of these two Fusaria may be compared with that of *Phytophthora infestans* on blight-resistant tubers as described by Müller & Börger (1941) where avirulent (*A*) strains caused a rapid necrosis of the tissues with which they were in contact and were prevented from further spread or fructification by this reaction on the part of the host; necrosis was less rapid in tissues infected by virulent (*S*) strains which were thus able to spread through the tuber and to fructify. Müller & Börger showed that growth, not only of *P. infestans* but also of other fungi (including *Fusarium caeruleum*, which was, however, less sensitive than *Phytophthora infestans*), was restricted in the necrotic areas. They concluded that a non-specific 'restrictive principle', which they termed a 'phytoalexin' had been formed by the host cells in response to the infection. The initial speckling which occurs when a cut tuber is inoculated with *Fusarium* spores is similar in appearance to the characteristic necrosis found in a tuber affected by blight, as noted by Gradinaroff (1943), who compared, specifically, the necrosis following inoculation with various *Fusarium* spp. (other than *F. caeruleum*) with that caused by *A* strains of *Phytophthora infestans* on resistant (*W*) varieties. Older lesions caused by Fusaria differ considerably from those caused by *P. infestans*, as might be expected from the greater ability of the former to grow saprophytically. The similarities of the earlier stages suggest, however, that the initial response of the tuber tissues to infection by Fusaria is similar to that due to *P. infestans* and is dependent on the sensitivity of the host cells to the pathogen.

A striking feature of the tuber diseases caused by Fusaria is the great variation in host susceptibility, which is influenced not only by variety but also by maturity (before and after harvesting), soil type, environmental conditions during the growing season, etc. In individual tubers the tissues near the surface are more resistant than those in the interior and the rose end is more resistant than the heel end (Boyd, 1952*a*). Cells which have responded to the stimulus of a nearby wound show marked resistance to infection by Fusaria. Such tissues are also more resistant to infection by *P. infestans*, as shown by Meyer (1940), who found that the browning reaction was more rapid and sporulation was reduced when inoculation was delayed for three days after wounding. Lansade (1950) has recorded that intumescences and tissues greened by exposure to light, in both of which reserve starch was hydrolysed, were resistant to infection by *Fusarium caeruleum*.

Availability of oxygen may be greater near a fresh wound surface than in deeper tissues, but, as the suberin layer has been shown (Thornton, 1939) to be largely impervious to gases, this factor cannot be responsible for the increase in resistance of cells under an intact wound periderm indicated by the restricted growth of lesions resulting from delayed inoculations. It would appear that the changes which occur in response to wounding are accompanied by an increase in the intrinsic ability of the affected cells to resist infection. Among other characteristics of such cells it may be noted that Belval & Laenffer (1946) found that polyphenol oxidase activity was much greater in cells of the normal or wound periderm (and of the vascular system) than in parenchyma cells; the oxidase was present in all cells but the substrate was localized to the tissues named. Johnston & Schaal (1952) found that chlorogenic acid, which can act as a substrate for polyphenol oxidase, accumulated in cells near a wounded surface. These observations are of interest as it has been suggested by Szent-Györgyi & Vietorisz (1931) that accumulation of quinoids in injured tissues due to polyphenol oxidase activity may be related to natural resistance to infection; Dufrenoy (1945) has also postulated that 'decompensated respiration' in affected cells may inhibit fungal growth.

Restriction of lesions caused either by *F. caeruleum* or *F. avenaceum* near the site of infection, or of larger lesions caused by *F. caeruleum*, was accompanied by the deposition of suberin on the host cell walls surrounding the infected tissues. It appeared that suberization in the small lesions was stimulated by the products of necrosis from host cells which had reacted violently to the infection, rather than by the direct action of the fungus mycelium which was present only in small amounts and localized in distribution. Restriction of *F. avenaceum* lesions which had achieved some size was frequently associated with changes in the adjacent host cells similar to those near a wound. These cells presumably acquired a resistance which prevented further spread of the fungus, but the typical response to infection found near the site of inoculation was not observed. Meyer (1940) described a similar effect in tubers resistant to infection by *Phytophthora infestans* and concluded 'that the cells are more sensitive (than in susceptible varieties) to some product of the parasite...and therefore respond by forming some toxic intermediate product without being in direct contact with the parasite and form a 'barrier' between the healthy and diseased tissues which the parasite cannot penetrate'.

The lack of response of the host cells to the approach of hyphae in spreading lesions caused by *Fusarium avenaceum* is surprising as the tissues can respond at times to the presence of lesions of all sizes by a wound reaction which restricts further development. However, toxic products diffusing from the lesion may cause a reduction in the sensitivity of adjacent cells similar to that found when tuber tissues are narcotized with alcohol or chloroform (Behr, 1949). The luxuriant growth of mycelium found in lesions caused by *F. avenaceum* must also lead to shortage of oxygen in the adjacent tuber tissues, another factor which may be responsible for the lack of host response. It is of interest to recall Schmidt's (1928) observation that lesions caused by *F. avenaceum*, if retarded for any reason, were unable to spread further even when conditions became favourable again.

The increase in susceptibility during the storage period noted by previous workers has been confirmed but it has not been possible to relate this to any particular change in the properties of the host tissues. It would appear, however, that the tissues of the freshly-harvested tuber are both sensitive to infection and able to respond by necrosis and suberin deposition; as the tubers mature, the reaction to infection decreases and eventually disappears. Early varieties are on the whole more susceptible to dry rot than are the maincrop varieties, a fact which suggests that the duration of the period of resistance may be related to that of the initial rest period, during which sprouting cannot be induced under any circumstances. The rate of suberization of wounds and of meristem development are known to decrease as the tubers mature, but little change could be detected in these properties during a period when considerable increase in susceptibility took place, nor was there any great difference between tubers of the resistant variety King Edward and the susceptible Doon Star. However, the rate at which tissues near a wound develop resistance to infection may not be related directly to the rate of wound periderm formation. Lansade (1950) has suggested that an alteration in the permeability of the membranes of the host cells and in the availability of sugars may be connected with the changes in susceptibility. Seasonal variations in the ability of the host tissues to respond actively to infection by the formation of substances restricting further growth of the fungus would appear to be another possibility, but a better understanding of the nature of the restrictive process is necessary before this hypothesis can be tested adequately.

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EFFECT OF TETRACHLORONITROBENZENE ON THE SPROUTING AND CROPPING OF POTATO TUBERS

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(With Plate 13)

Potato tubers dusted with a preparation containing 3% tetrachloronitrobenzene (TCNB) and kept in a nearly closed container in the laboratory showed reduced sprouting. The reduction was greater the lower the temperature and the earlier the application of the dust; it was less marked with tubers lying on a moist substratum. After long exposure, and especially at higher temperatures, sprouts of treated tubers tended to show a 'witch's-broom' appearance. The repressive effect of TCNB on growth rate disappeared as soon as the tubers were brought into ordinary air. To a certain extent the action of TCNB was antagonized by treatment of the tubers with ethylene chlorhydrin.

Replicated field experiments over three seasons, with four potato varieties, gave a measure of the check to bulking of the crop which is brought about by winter storage of seed tubers in presence of TCNB. This check was eliminated by airing the tubers before planting, the time necessary for this depending on conditions. An airing period of about 6 weeks, in an unheated greenhouse (apart from sun-heat) was found to be sufficient for this purpose.

The speed of emergence of the variously treated batches was closely correlated with the yields produced, especially in the earlier liftings.

The average number of shoots per plant was significantly increased by the dust treatment and this was reflected in a higher seed/ware ratio in the mature or late-lifted crop.

There was no evidence that the delay in sprouting brought about by TCNB was due to an effect on the respiration rate of the dormant tubers.

The concentration of active principle was materially diminished in treated clamps after the period (*c.* 5 months) of winter storage. Estimates were also made of the persistence of TCNB on exposed surfaces in still and in moving air.

The use of pentachloronitrobenzene for restraining the sprouting of potato tubers in clamps and for controlling, at the same time, rotting of the sprouts by *Rhizoctonia solani* has been described by Brown (1947). The following is an account of similar experiments, extending over three seasons (1948–51) with the more active derivative, tetrachloronitrobenzene (TCNB). The work was carried out, partly under laboratory conditions where the temperature was fairly well controlled, and partly in the open, with potatoes set up in clamps over winter under various treatments, and then put to grow in replicated plots in the ensuing summer. The effect of TCNB on the respiration rate of potato tubers was also determined under controlled conditions. The results will be set out here under these different headings.

LABORATORY EXPERIMENTS

The majority of these were carried out in tins (10 × 10 × 4 in.), with fairly tight-fitting lids. Into each of these twenty seed-size tubers (vars. Epicure, Great Scot, Arran Pilot, Home Guard) were placed, with or without a covering of a commercial preparation, Fusarex, which contains 3% TCNB as active principle. The standard dosage was at the rate of 7 g. of Fusarex (= 0·2 g. TCNB) per tin. The experiments were run at different temperature levels and the dust was applied for various periods. Results were assessed in terms of average sprout length (cm.), i.e. the total length of all sprouts present under any treatment divided by the number of sprouts. The experiments were replicated five times.

Table 1 records the behaviour of all four varieties in an experiment begun at the end of November and continued for 19 weeks. These varieties sprout readily as the storage season advances so that sprouts soon became long in the control series, especially at the higher temperature.

TABLE 1. *Effect of TCNB on sprout length at two temperatures*

		Time in weeks					
		6		12		19	
Variety	Av. temp. (° C.)	Control	TCNB	Control	TCNB	Control	TCNB
Epicure	13	5·0	1·0	20+	4·0	.	9·0
	4	2·5	0·5	10	1·5	15	2·5
Great Scot	13	4·75	0·75	20+	2·5	.	4·0
	4	2·5	+*	5·5	1·25	13	1·5
Arran Pilot	13	7·0	2·0	20+	3·0	.	3·5
	4	3·0	0·5	8·0	1·75	10	2·0
Home Guard	13	20+	+	20+	1·0	.	1·5
	4	5·5	+	13	0·5	20+	0·75

* Sprouts showing as small protuberances, < 0·25 cm.

The table shows that, in presence of TCNB, the sprouts are still quite short after 19 weeks' storage, especially at the lower temperature. The var. Home Guard was distinctly the most retarded and Epicure the least.

At both temperature ranges the sprouts of control tubers were heavily attacked by *Rhizoctonia solani*; those of treated tubers were free of fungal attack and were stouter. Treated batches showed more sprouts per tuber, especially so in var. Home Guard at the higher temperature where as many as fifteen sprouts might develop at an eye so as to give a 'witch's broom' appearance. Occasionally a side sprout might drill its way into or through the tuber, growing apparently unretarded while embedded in the flesh, and even a new tuber might arise inside the old one. This type of monstrosity was seen in Arran Pilot a few times, and in Home Guard quite frequently, after the tubers had been subjected to the dust at the higher temperature for about 4 months.

Table 2, which gives the behaviour of var. Home Guard at shorter intervals, shows clearly that the relative retardation of sprouts is greater at the lower temperature. Thus, after 7 weeks at 13·5° sprouts equalled 1·75 cm. in presence of TCNB, at which time the controls were 8·8 cm. long. At the lower temperature, after 16·5 weeks, the corresponding figures were 0·25 and 10 cm., thus indicating a more severe repression. This result falls in line with what was described by Reavill (1954) for repression of fungal growth by TCNB in relation to temperature.

The experiment of Table 2 as set up included a still higher temperature, viz. one fluctuating round 28° C. Results at this temperature, which are omitted from the Table, were completely vitiated by a terminal blackening of the sprouts, in controls and treated batches alike. As a result of killing at the apex, sprouts branched from the base, the process was repeated and in this way exaggerated witch's brooms were produced. No definite fungus was isolated from such dead apices, and the cause may be physiological. This effect was seen from time to time at lower temperatures also, it was more prevalent in the experiments of the first year than of the second, and it was less when tin boxes were replaced by less airtight boxes of cardboard. Bates & Dillon-Weston (1936) have described a similar effect seen when potatoes are sprouted in rather weak light.

TABLE 2. *Effect of TCNB on sprout-length of var. Home Guard at two temperatures*

Av. temp. (°C)	Treatment	Time in weeks 2	4	7	9	11·5	16·5
13.5	Control	1·5	3·5	8·8	16	20+	.
	TCNB	+*	0·75	1·75	2·0	2·5	.
5·5	Control	+	0·5	2·0	3·5	4·5	10
	TCNB	+	+	+	+	+	0·25

* As in Table 1.

Some of the effects described in the two preceding paragraphs are illustrated in Plate 13.

The batch of tubers used in Table 2 already showed some sprouting at the time the experiment was begun. These were sorted into two lots: (*a*) unsprouted, (*b*) with sprouts. The latter were removed, and the experiment run in duplicate with (*a*) unsprouted, (*b*) desprouted tubers. The data of Table 2 relate to the former. Those for the latter are omitted, as they were practically the same.

Under the same conditions, the relative retardation of sprout growth by TCNB is less the later the application of the dust. This is brought out in Tables 3 and 4, the latter of which is constructed from the data of Table 3.

All dusting treatments cut down sprout growth and the effect was greater the earlier it was applied, as shown by the steady increase in daily growth as one proceeds from treatment 2 to treatment 6.

Apart from the tendency of treated tubers to develop more sprouts than do the controls (p. 443) the effect of TCNB disappears as soon as the active principle is removed. Sprouts which have been retarded by the dust begin to grow, as soon as the dust is washed off, at the same rate as control sprouts at the same stage of development.

Table 3. *Sprout length of Home Guard tubers, dusted at varying times*

Treatment	Time in days						
	6	13	22	37	58	72	77
(1) Controls	0	1·0	1·75	2·85	5·2	10·5	12·3
(2) Dusted throughout	0	+*	+	0·75	2·0	2·5	3·2
(3) Dusted when sprouts just starting to grow	(D)+	0·45	0·5	1·5	2·6	3·1	3·8
(4) Dusted when sprouts av. 1 cm. long	.	(D) 1·0	1·5	2·0	3·0	4·2	4·8
(5) Dusted when sprouts av. 1·75 cm. long	.	.	(D) 1·75	2·5	3·1	4·9	5·9
(6) Dusted when sprouts av. 2·75 cm. long	.	.	.	(D) 2·75	4·1	6·1	6·8

(D) indicates when dusting occurred; *, as in preceding tables.

Table 4. *Daily growth of sprouts in relation to date of treatment*

Treatment (as in Table 3)	No. of days	Growth	Daily growth
1	77	12·3	0·16
2	77	3·2	0·042
3	71	3·8	0·054
	64	3·3	0·052
4	64	3·8	0·059
5	55	4·15	0·075
6	40	4·05	0·10

The effect of access of moisture to sprouting tubers is to increase growth rate, and to diminish the relative repression caused by TCNB. The following figures illustrate the magnitude of these effects:

	Controls (cm.)	TCNB (cm.)	Ratio TCNB/control
Tubers planted in dry sand in dark	3·4	0·5	0·15
Tubers planted in wet sand in dark	14	5·5	0·39

This result is in accordance with what is often seen in dusted clamps, viz. that a certain amount of sprouting is liable to occur round the base.

According to the work of Denny (1926) and others, stimulation of potato sprouting can be brought about by treatment of the tubers with various chemical substances, such as ethylene chlorhydrin, thiourea, etc. Pujals, Nylund & Kraut (1947) and Gandarillas & Nylund (1949) have shown that ethylene chlorhydrin hastens the emergence of tubers treated with the sprout-depressant methyl-ester of α-naphthalene acetic acid. Table 5 shows the effect of chlorhydrin treatment (one immersion in 3 % solution), with or without TCNB dusting, on sprouting of Home Guard tubers.

Ethylene chlorhydrin considerably reduced the depressant effect of TCNB without completely neutralizing it. There was also a suggestion that the growth rate of undusted sprouts was increased by ethylene chlorhydrin.

In March, tubers of Home Guard were taken from a clamp which had been dusted in the previous November. They were still unsprouted. Some were dipped in 3 % ethylene chlorhydrin, others simply dipped in water as a check to the removal of TCNB dust by the operation of dipping. Dusted tubers from the same clamp, without further treatment, and desprouted tubers from a control (undusted) clamp were included in the experiment. All batches were planted in moist soil in tins. The records taken 10 weeks later were as follows:

TABLE 5. *Effect of ethylene chlorhydrin on sprout length of dusted and undusted tubers*

	Time in days				
Treatment	12	28	55	70	82
Control	1·5	3·0	8·5	12	20+
Ethylene chlorhydrin	1·25	2·5	10·25	20+	.
TCNB	0	+	0·5	1·0	1·25
Ethylene chlorhydrin + TCNB	+	0·75	3·5	7·75	10·5

	Av. sprout length (cm.)
Controls	18·0
TCNB	8·5
TCNB dipped in water	9·3
TCNB dipped in 3 % ethylene chlorhydrin	14·3

The last-named treatment therefore considerably speeded up sprouting of tubers which had been retarded over winter. Whether this method is practicable on a field scale is doubtful, and as will be shown later there is a simpler method of achieving the same result.

FIELD EXPERIMENTS

These were carried out over three seasons (1948–51) at Slough and Harlington, on light to medium loam at the former, and on a heavy loam at the latter. The varieties were Home Guard, Arran Pilot, Epicure and Great Scot, the seed being either one-year grown or fresh from Scotland. In all cases it was substantially free from visible virus deterioration. The greater part of the seed stock was set up in small 2 cwt. clamps, in November, with or without TCNB, and immediately earthed up. The dust was applied more or less evenly, at the rate of 1 lb. of the commerical preparation per clamp, thus conforming with the rate of 10 lb. per ton which is the dosage recommended in practice. This will be referred to throughout as N. The remainder of the seed was placed in shallow boxes and allowed to sprout ('chit') over winter in a cool shed or greenhouse.

Some of the clamps, dusted or undusted, were left undisturbed until planting time. A number of control clamps were opened after 2 months, dusted at the standard rate and again earthed up. More often, a dusted clamp was opened after a variable time and the potatoes boxed and placed to chit in an unheated greenhouse. It was hoped that by airing, the active principle of the dust would be dissipated and sprouting would be under way at the date of planting. As a further variant, dusted tubers which were thus being aired were sometimes vigorously hosed before being placed to chit, in order to remove the bulk of the dust which was still present. Dosages of N/2 and 2N were also applied in some of the clamps.

As all the varieties used are prone to sprouting in storage, and as two of the winters were rather mild, the general experience was that undusted clamps, when opened in March–April, showed long sprouts (up to 20 cm.) towards the outside, and in the body of the clamp sprouts which were heavily diseased and often decapitated by *Rhizoctonia solani*. In contrast there was negligible sprouting in dusted clamps, except for a few potatoes round the base or on the southern shoulder. As a result dusted tubers were firm, in contrast to the shrivelled undusted tubers.

The cropping capacity of tubers which had received the variety of dusting treatments described above was compared with that of chitted and of undusted *desprouted* tubers. Control tubers, taken from undusted clamps, either had very long sprouts, which in practice could not be planted, or diseased ones. Such long or diseased sprouts were therefore taken off.

The standard planting unit was twenty tubers in a row 25 ft. long. As far as possible the twenty tubers of each unit were chosen so as to make up a certain weight. The various unit plots for each treatment were replicated, usually eight times, and randomized. The number of such plots in each year ranged from about 450 to 700. All diseased tubers were rejected, and it was probably for this reason that the percentage of blanks in the crop was extremely low. Thus in a planting of Home Guard there were only fourteen failures from over 6000 tubers planted, i.e. less than 0·25 %. Where such blanks occurred a correction was made in the figure for yield.

In each year plantings were made at two or three dates. The first of these was very early and ran the risk of being cut down by a late ground frost, but this did not in fact happen. The second planting was 3–4 weeks later, and its main purpose was to insure against accident to the first. A third planting was made in only one year.

For each planting there were as a rule three dates of lifting, one being early, at a time when the most advanced plots were yielding about 20 lb, which corresponds to *c.* 4 tons per acre. The second lifting was 2–3 weeks later, and the third when the tops had largely died down. At each lifting two to three replicates were used up. In this way the value of an 8-fold replication, for statistical treatment of data, was sacrificed; on the other hand it was considered more desirable to obtain a general picture of the build-up of yield throughout the season, under the various treatments.

Records were taken of the average date of emergence under each treatment and of the relative size of tops for a few weeks afterwards. The average number of stems per plant was also measured in some experiments of the series, and in these the ratio of ware to seed-sized tubers was also determined at the final lifting.

The records of these experiments were assembled into some twenty tables, fifteen of which, covering the first 2 years, were included in a Thesis presented by one of us (Reavill, 1950), a copy of which is deposited in the Library of London University. It is not proposed to detail these records here; it will be sufficient to give one table for purposes of illustration, and to state the broad conclusions which could be drawn from the records as a whole. Table 6 gives the yields (pounds per plot of twenty plants) at three liftings, the number of replicates used at each date being 2, 3 and 3 respectively.

TABLE 6. *First planting Epicure, Harlington* 1949. *Clamped* 10 *November* 1948, *planted* 29 *March*

No.	Treatment	Exposure to TCNB	Further treatment	Date of lifting 28 June	 15 July	 29 Aug.
1	Control, desprouted	None	None	12·5	17·8	28·8
2	Chitted	None	None	18·4	24·0	32·4
3	N	10 Nov.–29 Mar.	None	12·5	15·5	29·3
4	N/2	10 Nov.–29 Mar.	None	14·6	19·0	26·6
5	2N	10 Nov.–29 Mar.	None	12·0	17·1	28·5
6	N	24 Jan.–29 Mar.	None	15·1	17·8	28·0
7	N/2	24 Jan.–29 Mar.	None	13·8	18·3	27·3
8	2N	24 Jan.–29 Mar.	None	13·6	17·0	27·0
9	N	10 Nov.–10 Feb.	Aired 7 wks, washed	19·0	22·8	30·1
10	N	10 Nov.–10 Feb.	Aired 7 wks, unwashed	18·4	22·4	32·0

From these results the following conclusions are drawn:

(1) Chitted tubers and those which had been aired for 7 weeks after passing most of the winter in a dusted clamp were equal as regards cropping (2, 9, 10) and were ahead of all others.

(2) Desprouted controls, and tubers which had N and 2N dust treatment throughout (1, 3, 5) gave the lowest yield at the first lifting; the remainder, i.e. those with N/2 throughout and those which were not dusted until 24 January (4, 6, 7, 8) were intermediate.

(3) The differences between treatments were less obvious at the second and had largely disappeared at the final lifting.

(4) Washing of tubers before airing had no effect on their yielding capacity (9, 10).

A study of all the records available led to certain clear-cut and invariable results and to others which were irregular and which were therefore probably non-significant. These will now be summarized.

Invariably the best yields were given by tubers which had been chitted or which had been aired for some weeks after being clamped in presence of TCNB. An estimate of the number of weeks' airing necessary to remove the check to growth will be given later. Desprouted control tubers (i.e. from undusted clamps) and tubers taken straight from dusted clamps always gave smaller yields, especially at early liftings.

Table 7 gives a comparison between pairs of treatments in which the effect on yield was sometimes in favour of the one and sometimes of the other.

There was a suggestion that the depression in yield was greater the higher the dosage of dust enclosed in the clamp, but the difference was small and uncertain.

All three doses of TCNB tended to give smaller yields than the desprouted controls, but again there was little to choose between them. Washing of the dusted tubers, either before airing, or before the tubers were planted, had no measurable effect on yield.

TABLE 7. *Comparison of different treatments as affecting yield*

Treatment					
A	B	Total Comparisons	A>B	A=B	A<B
Control desprouted	N throughout	42	24	2	16
N throughout	N/2 throughout	34	13	3	18
N throughout	2N throughout	22	15	0	7
Treated tubers washed	Treated tubers unwashed	98	51	4	43

TABLE 8. *Effect of length of airing period on yield*

	Aired tubers		
Replications	Period of airing (weeks)	Yield (lb.)	Chitted tubers. Yield
3	13	36·1	36·8
6	11	40·0	41·7
13	9	27·9	30·0
20	7	28·0	27·5
14	5	33·0	38·0
17	3	24·5	30·4

In work of the kind just described, which aimed at combining disease control and the depressant effect of TCNB upon sprouting with satisfactory cropping of the tubers so treated, one used tubers which had been chitted in open trays as a standard of comparison. It became clear, however, that this was a variable standard as chitted tubers showed distinct differences in yielding capacity according to the temperature at which they had been held. Thus in the winter of 1950–1 it was found that tubers chitted in a cool shed until 1 February and then transferred to an unheated greenhouse (which warmed up considerably on sunny days) gave, when lifted early, a distinctly increased yield (39·8 against 28·7) over tubers kept in the cool shed throughout. In estimating the time necessary for airing dusted tubers in order that the check to growth may be substantially eliminated, it was important that the tubers being chitted and those being aired should be maintained under the same conditions.

Table 8 gives the results of various comparisons carried out as just described.

It is seen therefore that with the conditions of airing adopted, a period of about 7 weeks is required for the dusted tubers to become equal in yielding capacity to the chitted controls. Obviously no absolute value can be attached to this figure. At the time when tubers are taken from the dusted clamp, sprouting may not have visibly begun. Once they are exposed to the open air or very soon afterwards the depressant effect of the dust disappears (see p. 437) provided the vapour of TCNB is free to escape. There is, however, leeway to be made up and one might suggest that this could best be achieved by airing the tubers in a heated greenhouse. Possibly a more rapid effect could be obtained by airing in a warm darkened room, provided there was a free circulation of air. The effect of etiolation which is to be avoided in overwinter chitting might well be desirable when the aim is to bring on sprouting as quickly as possible.

Percentage emergence and relative size of plant

Throughout the work the effect of treatments upon date of emergence was recorded, and it was found that the earlier the emergence the greater the crop yield at the first, and probably also at later, liftings. Table 9 is one of a number which illustrated this relationship. In addition to a count of emergence, the early growth of the plants was assessed by eye into four grades, 1–4, in increasing order of size. Such a record is also included in the table.

TABLE 9. *Effect of treatments on emergence, early growth and yield of potato (Great Scot), planted 29 March*

Treatment	% emergence on 5 May	Relative size 12 May	Yield 14 July (lb. per twenty plants)
Control desprouted	19	3	13·8
Chitted	96	4	17·6
N throughout	4·5	1	11·3
N/2 throughout	13	2	13·3
2N throughout	3	1	12·0
N from 24 January	33	3	14·6
N/2 from 24 January	26	3	13·2
2N from 24 January	9·5	2	12·0
N, aired 7 wks, washed	95	4	20·5
N, aired 7 wks, unwashed	89	4	15·4

The general parallelism of all three sets of records is obvious in Table 9. This type of result has been so uniform that one can confidently forecast which treatments in a batch will lead at an early lifting.

Number of shoots per plant in relation to treatment

Counts were made of the number of separate shoots in the various plots, mainly at the time when the tops were about half-grown. In a number of plots these figures were checked by counts at the time of lifting.

Table 10 gives the summarized records for two varieties in 1949 and two in 1950. Altogether there were, with each variety, eight to ten variations in the dusting treatment applied (e.g. N, N/2, 2N; airing for various periods; washing or not washing). As all these dusting treatments had the same general effect on numbers of shoots, they are taken together in the table and the overall average number given.

With the exception of Epicure all the averages shown are significantly different, the degree of significance much exceeding 100:1. With Epicure the only significant difference is that between 'dust treatment' and 'chitted' ($P=0\cdot05$). The difference between 'control desprouted' and 'chitted' lies one way with two varieties and inversely with the other two and may have been influenced by seasonal differences. The presence of *Rhizoctonia solani* on sprouts of 'control desprouted' has probably also some effect. However, the important difference, which is shown distinctly by all four varieties, is that the dust treatments increase the number of sprouts as compared with the chitting process. It is no doubt for this reason that when the crop is allowed to run to maturity the ware/seed ratio is higher in the 'control desprouted' and chitted batches than in those which have come from tubers stored in presence of TCNB. Table 11 gives the summarized results for two varieties. As in Table 10, a number of dust treatments (thirteen for Arran Pilot, eleven for Home Guard) were examined in this respect, and as they all behaved in substantially the same way as concerns ware/seed ratio, the results of these treatments are combined into one overall average.

TABLE 10. *Effect of treatments on number of shoots per plot of 20 plants*

Treatment	Great Scott	Epicure	Home Guard	Arran Pilot
Control desprouted	94·8 ± 2·7	78·0 ± 3·5	76·3 ± 1·5	81·0 ± 1·9
Chitted	82·0 ± 1·6	71·1 ± 2·9	92·7 ± 1·8	97·8 ± 2·7
Dust treatments	128·4 ± 3·4	86·5 ± 4·1	118·5 ± 4·1	128·0 ± 4·7

TABLE 11. *Weight of ware per unit weight of seed in the crop*

Treatment	Arran Pilot	Home Guard
Control desprouted	3·1 ± 0·29	2·4 ± 0·36
Chitted	2·8 ± 0·36	1·7 ± 0·19
Dust treatments	2·2 ± 0·51	1·1 ± 0·27

The ware/seed ratio diminishes downwards in Table 11 for both varieties, but none of the differences is significant for Arran Pilot; for Home Guard, the difference between 'Dust treatments' and 'Control desprouted' is significant at more than the 20:1 level of significance, for 'Dust treatments' against 'Chitted' the difference almost reaches that level. The general trend of the results suggests that the differences observed are real, especially in the light of the effect on shoots pointed out in Table 10. It appears therefore that the dusting treatment is liable to reduce the proportion of ware-sized tubers in a crop; on the other hand it may be suggested as a means of increasing the yield of seed-sized tubers in the seed-producing areas.

EFFECT OF TCNB ON RESPIRATION RATE OF POTATO TUBERS

It has been shown by Appleman & Miller (1926), Whitehead (1934) and others that the rate of respiration of potato tubers falls soon after harvesting, remains at a low constant value for some time, and then increases when sprouts begin to develop. Miller (1933, 1934, 1935) and Miller, Guthrie & Denny (1936) showed that tubers treated with various bud-forcing chemical substances had an increased respiration rate even before any development of buds was visible. On the other hand the effect of certain primary alcohols, which likewise acted as breakers of dormancy, was to depress respiration rate. It does not seem, therefore, that acceleration of sprouting is related in any obvious way to intensity of respiration. Nevertheless it was considered desirable to examine whether the sprout-retarding effect of TCNB was accompanied by any reduction of respiration rate.

TABLE 12. *Rate of respiration of Home Guard and Majestic tubers. Experiment set up* 13 *September*

	Home Guard				Majestic			
	Control		Treated		Control		Treated	
Date	Sprout length	CO_2	Sprout length	CO_2	Sprout length	CO_2	Sprout length	CO_2
5 October	0	8·6	0	8·2	0	7·4	0	6·9
17 October	0	7·8	0	8·0	0	6·3	0	6·4
26 October	0	8·2	0	7·7	0	5·6	0	5·9
8 November	+	9·5	0	8·2	0	5·2	0	5·4
14 November	++	12·0	0	7·9	0	5·9	0	5·3

+, sprouts just visible as pin-points; ++, sprouts begun to lengthen but < 0·25 cm.

The work in this connexion was carried out with Majestic and Home Guard tubers, two varieties which have respectively a long and a short period of dormancy under ordinary conditions of winter storage. Twenty tubers, free from any obvious injury and of approximately uniform size, were placed in each of a series of tins with lids, half of them with 0·2 g. TCNB (in the form of 7 g. of 3 % dust), the other half being controls. Storage was in a cool room, with temperature range 0–12° C. At intervals the various lots of tubers were transferred to a desiccator which was connected up with a gas-analysis apparatus and a stream of CO_2-free air, regulated and measured by means of a flow-meter, drawn over the potatoes and into the analysis apparatus. The amount of CO_2 evolved under standard conditions was measured by the usual double titration method, with phenolphthalein and methyl red as indicators. The standard run for each batch was one of 5 hr., after which the potatoes were put back into the storage room. From time to time blank runs were carried out in order to determine the correction to be made for CO_2 diffusing into the apparatus. Table 12 gives the records obtained during the early winter of 1950–1, and these were similar to the results of the preceding year. The figures in the table represent weights of CO_2 in mg. given off in 1 hr. by 1 kg. of potato tubers.

There is clearly no suggestion that respiration is affected by the dust treatment, so long as sprouting is still in abeyance. As soon, however, as the beginning of sprouting could be detected, as with the control Home Guard tubers in the above Table on 8 November, a distinct increase of respiration rate was shown. The repression of sprouting brought about by the dust treatment cannot be linked up with any repressive effect of TCNB on respiratory activity.

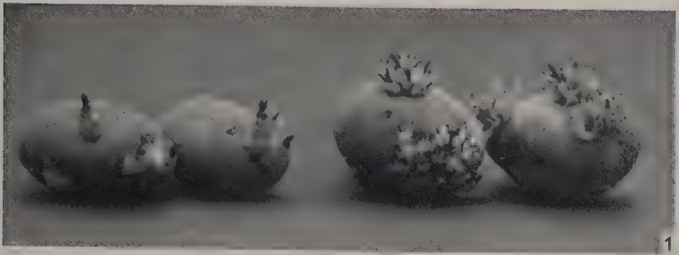
PERSISTENCE OF TCNB ON SURFACES

In general, one application of TCNB at the standard rate N (see p. 440) is sufficient to repress sprouting over the winter storage period, November–March. After this, when the weather becomes warmer, development of sprouts begins. This may be due to the dust causing less retardation at a higher temperature (see p. 437) or to the disappearance in the meanwhile of most of the active principle. When the dust coating the tubers is examined in March its appearance is the same as at the time of application, but the odour, which is characteristic, is distinctly less. This suggests that the active principle is much reduced in amount, a conclusion which is supported by the following experiment.

Two clamps which had been treated in November with the normal dose (N) were completely bared of soil and straw in March. At this time, apart from a few sprouts showing on the south side and round the base, all tubers were unsprouted. One clamp was taken down and rebuilt with a second dusting at the standard rate. Both were then restrawed and resoiled. When finally opened at the end of June, a distinct difference was seen. In the once-dusted clamp there were long interwoven sprouts to a depth of about five tubers; in the centre, sprouts were shorter, reaching up to a maximum of about 7 cm. In the other there were no sprouts exceeding 2 cm. The second dusting therefore had a marked effect, which would not have occurred if a good proportion of the first applied dust had been present in March.

By using the Petri-dish technique described by Reavill (1954), in which the effect of a given amount of TCNB, placed as a film in the lid of the dish, is tested on the germination of *Botrytis* spores, it is possible to detect the presence of as little as 0·0001 g. of TCNB. A similar degree of sensitiveness is shown by a colour test described by Auerbach (1950). Both methods gave a positive reaction when the dust present in March on the surface of clamped potato tubers was tested. Neither method, however, is adapted for quantitative estimations.

Films of TCNB (0·1 g.) were deposited on Petri-dish lids (8·5 cm. diameter). Some were exposed outside (mean temp. 17° C.) but protected from rain; others in the laboratory (mean temp. 21°). Tested by the two methods available, the films placed outdoors gave a positive reaction after 7 days but none after 9 days. Those indoors continued to give a positive reaction for at least three months. This rough experiment suggests that wind is the most potent factor in hastening the disappearance of TCNB. In clamps there is probably considerable variation in the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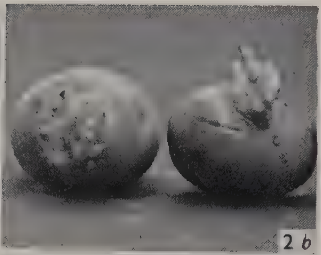
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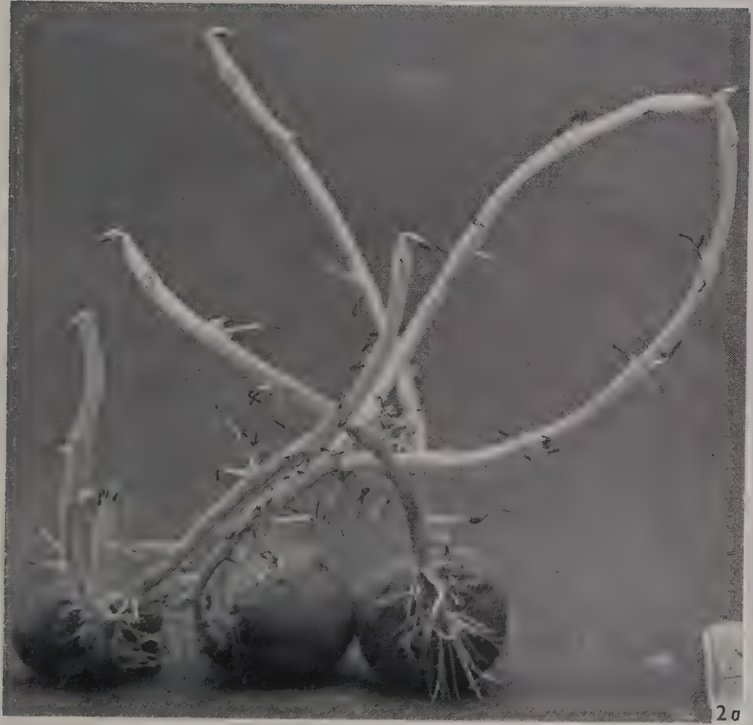
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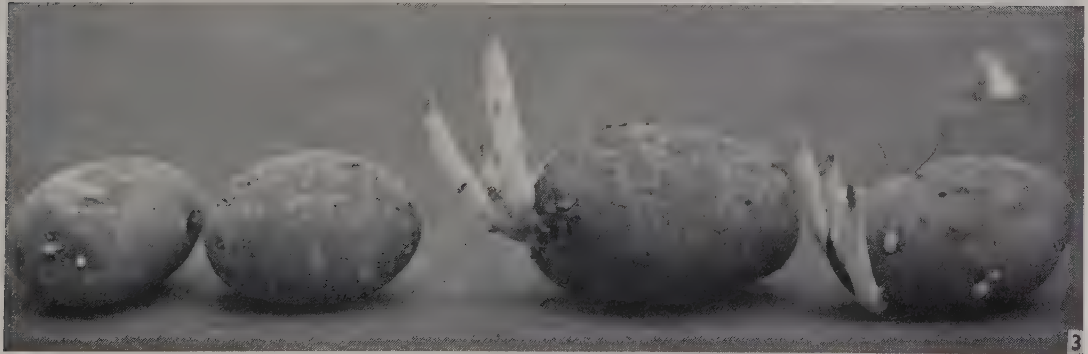
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Brown and Reavill—*Effect of TCNB on sprouting and cropping of potato tubers*

extent to which air circulates, according to the manner in which the clamp is soiled up; the possibility of some microbiological decomposition within the humid atmosphere of the clamp is also not excluded.

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EXPLANATION OF PLATE 13

Appearance of potato tubers stored for 9 weeks at different temperatures, in presence or absence of tetrachloronitrobenzene.

Fig. 1. 23–32° C. *Left*: Control; short sprouts with black tips. *Right*: TCNB; short sprouts with black tips; pronounced witch's-broom effect.

Fig. 2. 10–17° C. 2*a*: TCNB; short sprouts with occasional black tips. 2*b*: Control; long sprouts, roots developed, tubers shrivelled.

Fig. 3. 0–12° C. *Left*: TCNB; sprouts just showing. *Right*: Control; sprouts averaging about 4 cm.

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EFFECT OF CERTAIN CHLORONITROBENZENES ON GERMINATION, GROWTH AND SPORULATION OF SOME FUNGI

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(With 4 Text-figures)

When grown in an atmosphere saturated with tetrachloronitrobenzene (TCNB) vapour, *Botrytis cinerea* showed retarded germination and colony growth and its sporulation was completely suppressed. Retardation of growth was greater on dilute or acidified media and at lower temperatures.

Similar effects were shown by *Rhizoctonia solani*, *Phytophthora parasitica*, *Mucor hiemalis* and *Trichoderma viride* but none of these was as sensitive as *Botrytis cinerea*, especially as regards linear growth. Of two strains of *Fusarium caeruleum*, one was much more sensitive than the other, both in linear growth and sporulation. *Pythium deBaryanum* was unaffected in its growth rate.

TCNB, as a growth repressant, is fungistatic without being fungicidal.

With fungi which are sensitive, tetrachloronitrobenzene was more effective than the penta-derivative, except for *Trichoderma viride* where the converse applied.

Introduction

The value of chloronitrobenzenes for the control of certain fungal diseases of crop plants has been tested over a number of years at the Slough Field Station of the Imperial College. A preparation containing pentachloronitrobenzene (PCNB) was first used as a possible control measure for club-root disease but was early discarded for this purpose in favour of the trichlorodinitroderivative (Smieton, 1939). In the meantime it had been found that PCNB gave promise of controlling the *Botrytis* disease of lettuce. Work in this connexion has been reported on by Brown (1935), Smieton & Brown (1940), Brown & Montgomery (1948) and Last (1952), a third derivative, viz. tetrachloronitrobenzene (TCNB) being also used in the more recent investigations. Substantial control of the *Botrytis* disease was given, better with TCNB than with PCNB, and both substances considerably reduced damping-off when this was caused by *Rhizoctonia solani*. On the other hand, damping-off caused by *Pythium* spp. was unaffected and there was no or slight control of mildew (*Bremia lactucae*). It was also shown that both PCNB and TCNB, if dusted on tubers in clamps, very materially reduced the damage to sprouts caused by the solanaceous strain of *Rhizoctonia solani* (Brown, 1947).

In recent years TCNB, applied as a dry dust to potatoes at the time of lifting, has given a good control of the dry-rot disease caused by *Fusarium* spp. Papers by Foister & Wilson (1943), Boyd (1947) and Foister & Wilson (1950) give an account of the co-operative work on this disease by the Agricultural Research Council and the Department of Agriculture for Scotland.

The direct effects of chloronitrobenzenes on fungal growth have so far been little studied. Roy (1947), working in this laboratory, made a comparison of PCNB, TCNB, Bordeaux mixture and Shirlan. The following is a fuller account of the action of chloronitrobenzenes, and of TCNB in particular, on the germination, linear growth and sporulation of a number of fungi.

MATERIALS AND METHODS

Most of the detailed work was carried out with a strain of *Botrytis cinerea* Pers. from lettuce. Other fungi used were the following:

Mucor hiemalis Wehmer. Culture from Commonwealth Mycological Institute.

Fusarium caeruleum (Lib.) Sacc. Isolated from potato by Dr Wilson, Sutton Bonington. This will be referred to as strain I.

Fusarium caeruleum (Lib.) Sacc. From potato at Harlington, Middlesex (strain II).

Phytophthora parasitica Dastur. From tomato plant.

Pythium deBaryanum Hesse. From lettuce seedling.

Rhizoctonia solani Kuhn. Three strains, from (*a*) potato, (*b*) cabbage seedling, (*c*) lettuce seedling.

Trichoderma viride. Stock culture in laboratory.

All the cultures named, except *Mucor*, *Fusarium* strain I and *Trichoderma* had been recently isolated by various workers in the Department.

The only method which calls for description is that which concerns the application of the chemical substance. Both PCNB and TCNB are almost insoluble in water and both have a low but definite vapour pressure, that of PCNB being lower in the ratio of about 1:4. It was important therefore to determine how far activity on fungal growth was due to action of the vapour or to contact or near-contact with the solid substance in the culture medium. For this purpose the following comparison was made:

(1) 0·3 g. of the finely ground crystals of TCNB was spread evenly over the surface of an agar medium in a Petri dish of 8·5 cm. diameter.

(2) 10 ml. of a 3% solution of TCNB in acetone was evaporated to dryness in the lid of a similar dish. This gave a film of crystals, 0·3 g. in weight, which was not easily shaken off the glass. Control experiments in which 10 ml. of the solvent was evaporated showed that there was no residue which had a detectable effect on fungal growth.

One ml. of a standard suspension of *Botrytis* spores was now spread over the surface of each plate (potato agar). The following figures were obtained after 4 hr. incubation at 20° C:

Percentage germination:	
Controls	88·45 ± 0·69
Vapour only	25·7 ± 3·5
Crystals on medium	24·8 ± 4·0

These results show that there is considerable slowing down of germination and that both methods of application have substantially the same effect. As the vapour method was much the more convenient, it was used throughout the work.

In applying this method it was important to ensure that the vapour pressure was kept constant throughout the experiment, and this meant that temperature must be kept constant and the film of crystals not allowed to evaporate away. Many of the experiments were carried out by exposing inoculated agar-coated slides in tins (10 × 10 × 4 in.) with ordinary lids, and with this equipment films of 0·6, 0·3, 0·15 g. deposited on a glass plate of 8·5 cm. diameter gave equal repressive effects in experiments at 20° C. and lasting over 3 days. It was considered therefore that a film of 0·3 g. was adequate for the purpose and experience later showed that this was true also for experiments of much longer duration. With slides exposed in 8·5 cm. Petri dishes, the quantity used was 0·1 g., even though it was found to be no more effective than 0·01 g.

Furthermore no difference in effectiveness could be detected whether the chemical film was introduced into the container at the same time as, or 2 days ahead of, the introduction of the fungus-bearing medium. On the basis of such preliminary experiments, the standard procedure was to place the medium, bearing spores or mycelium, into the containers at the same time as the chemical film. The latter was 0·3 or 0·1 g. according as exposure took place in a tin or in the much smaller volume of a Petri dish. When containers were opened for purposes of record, care was taken that all the members of a series were treated uniformly in this respect.

EFFECT ON SPORE GERMINATION

Spores of *Botrytis cinerea*, taken from cultures 10–14 days old, were mainly used for this work. These were obtained by rubbing off the aerial mycelium under a layer of water, removing mycelium and coarser debris by filtering through fine muslin, breaking up the spore clumps by shaking, and washing a number of times on the centrifuge. The density of suspension was finally adjusted to give *c.* 25 spores per microscope field (10 × eyepiece, 1/6-in. objective).

Table 1 gives the results of a typical experiment. TCNB was used in two forms, as a film deposited from solution in the manner already described, and as the equivalent amount of a commercial preparation 'Fusarex' (3 % TCNB) in which the active principle is finely ground up with an inert filler. Average germ-tube length was measured by eye, in terms of average spore length. This method allows of quick determination and with practice it is reasonably accurate so long as the germ-tubes are fairly short, i.e. do not exceed ten spore lengths. The figures in Table 1 are based on counts of twenty microscope fields, totalling approximately 500 spores. The medium was 1 % malt agar and the temperature 20° C.

TCNB in either form strongly retards germination, as measured in either of the two ways adopted, but it does not stop it. Complete germination is eventually obtained in all cases.

TABLE 1. *Percentage germination and germ-tube length of* Botrytis cinerea *at various time-intervals*

		Time (hr.)						
	Treatment	2	4	7	10	16	19	24
Percentage germination	0·3 g. TCNB	2·5	29	68	80	95	98	98
	10 g. Fusarex	1·5	23	57	71	86	89	97
	Control	16	79	97	100	·	·	·
Average germ-tube length	0·3 g. TCNB	0·125	0·25	1·25	3·0	6	9	10+
	10 g. Fusarex	0·125	0·125	1·0	2·0	4	6	9·5
	Control	0·25	1·25	8·0	10+	·	·	·

The repressive effect of Fusarex powder is somewhat greater than that of TCNB film, as is seen in Table 1, and this result has been repeatedly confirmed. Thus in eight experiments, in each of which a duplicate comparison was made, the effect of Fusarex powder was greater in fifteen of the comparisons and equal in the remaining one. The difference was significant at the 20:1 level. Every precaution was taken to avoid disturbance of the dust, though the possibility that fine particles may have settled on the culture slides was not excluded. The effect may be due to the fineness of the powder which would allow more rapid volatilization than would take place from the crystalline film.

Spores of *Botrytis* which have germinated in presence of TCNB vapour show a qualitative difference as compared with the controls. Fig. 1 illustrates this point. Treated spores produce stout stumpy germ-tubes which branch early, whereas those of the controls are thinner and usually do not branch until they reach eight to ten spore lengths.

Within limits, the retarding effect of TCNB vapour is greater the weaker the medium. Fig. 2 is typical of the curves obtained in six experiments of this kind. The amount of growth shown in the controls also diminishes with concentration of medium but this effect is magnified in the presence of TCNB vapour, as shown by the fact that the curves marked II are more widely spaced out than are the controls (I). This effect was shown with media ranging from 3 % malt agar down to plain agar.

Comparisons between potato agar at pH 6·4 and at pH 4·2 showed that the retarding action of TCNB was increased by higher acidity. Figs. 3 and 4 illustrate this point, the former with regard to percent. germination and the latter to average

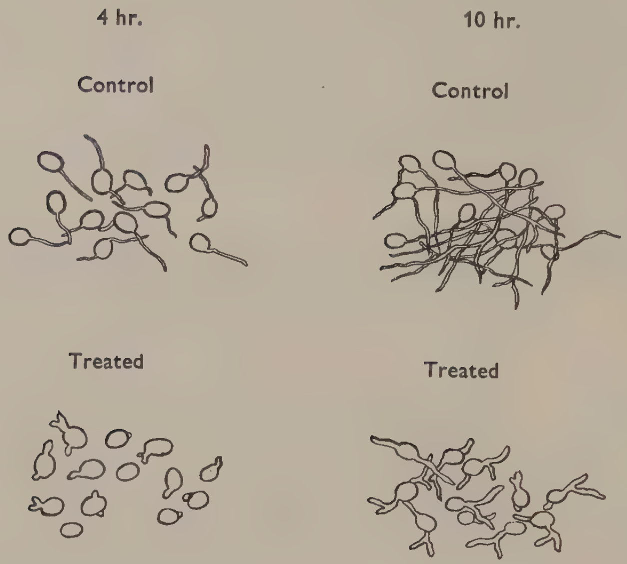
4 hr.
10 hr.
Control
Control
Treated
Treated

Fig. 1. Spores of *Botrytis* germinating in presence or absence of TCNB.

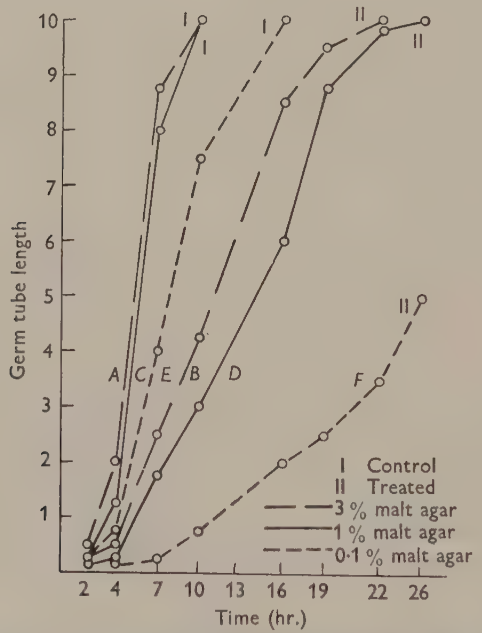

Fig. 2. Effect of TCNB on average germ-tube length of *Botrytis* spores at different concentrations of medium.

germ-tube length. This effect is more pronounced than that of concentration of medium already described.

Similar experiments on malt and lettuce leaf-extract agars with spores of *Trichoderma viride* gave the same type of result as with spores of *Botrytis cinerea*. Spores of the former are less sensitive to the action of TCNB.

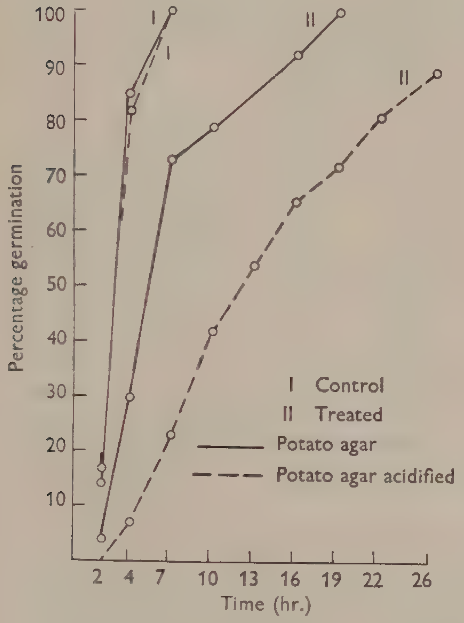

Fig. 3.

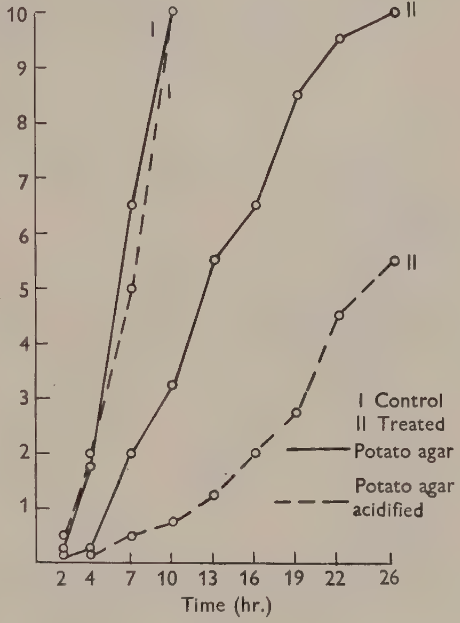

Fig. 4.

Fig. 3. Effect of TCNB on percentage germination of *Botrytis* spores on ordinary and acidified potato agar.

Fig. 4. Effect of TCNB on average germ-tube length of *Botrytis* spores on ordinary and acidified potato agar.

LINEAR GROWTH

Experiments were carried out in 8·5 cm. Petri dishes, each containing 25 ml. agar medium, and all the fungi listed on p. 449 were used. As some of these did not sporulate freely, a mycelial inoculum cut with an 0·5 cm. corkborer from the margin of a young culture was placed at the centre of the plate, and the colony diameter recorded was corrected for the diameter of the inoculum. TCNB was applied at the rate of 0·1 g. per Petri dish, the film being deposited on the lid. Experiments were run in quadruplicate.

Table 2 shows the general behaviour of the eight fungal isolates as grown on potato agar. Diameters of the control colonies after 9 and 15 days are omitted from the table, as the cultures by that time had filled the plates.

The effect of TCNB on linear growth varies considerably from fungus to fungus. At one end of the series is *Pythium* which is quite unaffected: *Phytophthora*, *Trichoderma*, *Rhizoctonia* and *Fusarium* I are definitely retarded, *Fusarium* II more so and *Botrytis* most of all. Even after 33 days the retardation of *Botrytis* was so pronounced that the treated colony was only 3·2 cm. in diameter, in contrast to the control which had nearly filled the plate in 6 days.

TABLE 2. *Effect of TCNB on linear growth of fungi*

	Time (days)					
	3		6		9	15
Fungus	Control	TCNB	Control	TCNB	TCNB	TCNB
Botrytis cinerea	5·0	+*	7·1	+*	0·2	0·7
Fusarium caeruleum I	4·0	2·2	6·7	4·3	5·6	6·4
Fusarium caeruleum II	3·5	0·7	6·3	1·7	2·5	3·8
Rhizoctonia solani	6·1	2·0	8·5+	3·5	4·9	6·0
Pythium deBaryanum	8·0	7·8	8·5+	8·5+	8·5+	8·5+
Phytophthora parasitica	5·3	3·1	7·0	5·7	7·8	8·5+
Trichoderma viride	7·0	3·6	8·5+	5·3	8·0	8·5+
Mucor hiemalis	6·3	4·0	8·5+	6·0	7·7	8·5+

* Growth had begun, but it was not visible macroscopically beyond the margin of the inoculum.

The strain of *Rhizoctonia* used in Table 2 was isolated from potato. In further experiments it was found that the isolates from cabbage and lettuce behaved similarly.

The greater response of *Fusarium caeruleum* II, which was a freshly isolated strain, than of *F. caeruleum* I which had been in culture for at least two years, should be noted. It may have behaved differently from the beginning; on the other hand strain I may have arisen undetected as a saltant in culture. The development of resistant forms in culture will be dealt with in a later paper.

In addition to the quantitative effects shown in Table 2, qualitative effects also appear. Instead of the normal circular outline seen in the controls, retarded colonies are lobed and have reduced formation of aerial mycelium and spores. Treated cultures of *Trichoderma*, though not greatly retarded, are characterized by a skin-like growth which often shows zonation. Sporulation is suppressed with *Botrytis* and considerably reduced with *Fusarium* and *Trichoderma* (see Section following). Sclerotium formation by *Rhizoctonia* is also reduced.

In the experiment of Table 2 the treated cultures were subjected to the vapour of TCNB from the beginning. Table 3 gives the behaviour of *Botrytis* cultures which were placed in TCNB vapour at various times after inoculation. The cultures were on potato agar at 20° C.

The increase in diameter made in 15 days after application of treatment (last column less 2nd column) was 0·8, 1·1 –, 1.0, 1·2, 1·1 + for the respective treatments as listed downwards in the table. Thus repression of linear growth is largely independent of the state of development of the colony at the time of applying the TCNB.

To determine whether TCNB has a fungicidal or merely fungistatic effect, colonies of *Botrytis cinerea*, *Fusarium caeruleum* I and *Rhizoctonia* were grown for varying periods in TCNB and then removed from the vapour. Table 4 gives the diameters 3 and 6 days after removal. The TCNB treatment was applied at the time of inoculation.

TABLE 3. *Effect of TCNB on* Botrytis *cultures when applied at different times*

Date of treatment	Diam. of colony at treatment	Time in days after treatment: 3		9	15
		Control	TCNB	TCNB	TCNB
At inoculation	0	4·8	0	0·3	0·8
1 day after inoculation	+	5·7	+	0·5	1·1
2 days after inoculation	2·6	6·4	2·8	3·0	3·6
4 days after inoculation	4·8	8·5+	4·9	5·2	6·0
6 days after inoculation	7·4	8·5+	7·6	7·9	8·5+

TABLE 4. *Linear growth after exposures of different length to TCNB*

Fungus	Duration of exposure (days)	Size of colony after exposure	Time in days after exposure: 3	6
Botrytis cinerea	0	0	5·2	7·5
	3	+	4·3	6·4
	6	+	4·7	6·8
	12	0·5	5·0	7·3
	24	2·1	6·4	8·5+
Fusarium caeruleum I	0	0	3·5	6·2
	3	1·9	4·8	7·6
	6	3·8	6·5	8·5+
Rhizoctonia solani	0	0	6·3	8·5+
	3	2·2	7·6	8·5+
	6	3·4	8·5+	8·5+

The increases in diameter shown by *Botrytis cinerea* in 3 days after removal from treatment of 3, 6, 12 and 24 days' duration are 4·3, 4·7 –, 4·5 and 4·3 respectively, as against 5·2 for the untreated control. There is therefore a small carry-over of the effect, but this does not persist, as is shown by the equal growth made by all the cultures between the 3rd and the 6th day after removal from treatment. As far as the data go, the same conclusions apply to the other two fungi. It is possible that the lag effect disappears much sooner than in 3 days, and that in fact it vanishes as soon as the active substance has vapourized from the treated culture.

A study of all the data available shows that the average increment of diameter of *B. cinerea* at 20° C. varies between 1·0 and 1·5 cm. per day, according to the stage at which measurement is made. The corresponding growth in presence of TCNB is 0·05 in the early stages, rising to 0·1 later. There is a suggestion of adaptation by the fungus in the last two figures given, but even at the higher growth rate there is a repression of more than 90 % as compared with the untreated control. The degree of repression shown during germination (e.g. in Table 1) is much less than this, so that germination appears to be less affected by TCNB than is mycelial growth. It must be remembered, however, that the period of exposure in experiments on mycelial growth extended over days, whereas in the germination studies the significant period was a few hours. It is probable that the short period required by spores of *B. cinerea* for germination is not sufficient to allow the vapour of TCNB to reach saturation in the nutrient medium, and still less within the fungal cells; in other words germination may be well advanced before the active substance comes fully into operation. On the evidence available one cannot conclude that germination is less affected than is mycelial growth.

TABLE 5. *Linear growth as affected by pH in presence or absence of TCNB*

			Time in days			
			6		15*	24*
Fungus	Medium	pH	Control	TCNB	TCNB	TCNB
Botrytis cinerea	Potato	6·2	7·1	+	0·7	1·6
		4·6	6·7	+	+	+
Rhizoctonia solani	Potato dextrose	6·4	8·5+	3·5	6·0	7·6
		4·6	7·5	2·6	5·5	6·8
			Time in days			
			3		9*	15*
Fusarium caeruleum I	Potato dextrose	6·4	4·0	2·2	5·6	6·4
		4·6	3·5	0·3	3·0	4·6
Pythium deBaryanum	Potato dextrose	6·4	8·0	7·8	8·5+	8·5+
		4·6	7·1	5·2	6·1	7·7
Trichoderma viride	Malt	4·8	7·0	3·6	8·0	8·5+
		3·2	6·7	2·1	4·4	6·2

* Controls omitted from table, as being 8·5+ in all cases.

The effect of TCNB on linear growth was tested for the various fungi on several media, viz. malt, potato, potato dextrose, lettuce leaf extract and plain agars. The amount of repression shown by each fungus was not materially affected by the medium except that it was greater with malt and plain agars. The greater sensitiveness on plain agar is to be ascribed to the low nutritive value of this medium (cp. Fig. 2). The malt agar used had a pH of 4·8 as compared with pH 6·2 for the other media, and this higher acidity is probably the cause of the greater response shown (cp. Figs. 3 and 4).

The effect of pH of medium is shown in Table 5 for five fungi on three media, at two pH values of the latter. The figures combine the results of four experiments.

Increased acidity invariably increased the effectiveness of TCNB. Even *Pythium deBaryanum* showed some repression on the acidified medium, but the effect was most pronounced with *Botrytis cinerea* which had just begun to grow out on acidified potato extract after 24 days' treatment. It may be that TCNB is intrinsically more active in presence of an acid, or alternatively that it is more soluble in acidified media. The solubility of TCNB (and PCNB) in aqueous solution is extremely low, being less than 0·25 mg. in 100 ml., and this fact, combined with its appreciable volatility, renders any accurate determination of its solubility very difficult.

The effect of temperature is shown in Table 6, which combines the result of four experiments.

TABLE 6. *Linear growth at two temperatures in presence or absence of TCNB*

			Time in days					
			6		15		24	
Fungus	Medium	Temp. °C.	Control	TCNB	Control	TCNB	Control	TCNB
Botrytis cinerea	Potato	20	7·0	+	8·5+	0·7	.	1·6
		10	4·5	0	8·5+	0	.	+
Rhizoctonia solani	Potato dextrose	25	8·5+	3·5	8·5+	6·0	.	7·6
		10	5·3	1·7	8·5+	4·0	.	6·3
Fusarium caeruleum I	Potato dextrose	25	6·7	4·3	8·5+	6·4	.	8·0
		10	2·4	+	5·9	1·8	8·0	3·6
Pythium deBaryanum	Potato dextrose	25	8·5+	8·5+	.	.	.	.
		10	3·5	1·6	5·1	3·2	8·0	6·9
Trichoderma viride	Malt	25	8·5+	5·3	.	8·5+	.	.
		10	3·1	+	5·6	2·3	8·5+	4·8

It is clear that the effect of TCNB is increased at a lower temperature, and *B. cinerea* again shows this result to a high degree. At a lower temperature the vapour pressure is less and the solubility of it is probably decreased. There are therefore good grounds for stating that TCNB is intrinsically more active at lower temperatures.

One can say, in summary, that the repressive effect of TCNB on fungal growth accords with the general rule of being greater the lower the growth potential, i.e. at a low temperature or on a weak medium. It is also accentuated by acidity of the culture medium.

SPORULATION

Throughout the work of the preceding section it was observed that treatment with TCNB had the effect of reducing sporulation. This effect was first noted by Smieton & Brown (1940) in connexion with the dusting of lettuce frames with a PCNB preparation as a control of *Botrytis* disease. Dusting resulted in the suppression of sporulation on dead lettuce material within the frame, so that the young plants were much less exposed to infection.

Experiments were carried out with Petri-dish cultures of *Botrytis cinerea*, *Fusarium caeruleum*, both strains, and *Trichoderma viride*. Estimations of spore numbers were made at weekly intervals in the following manner. To each Petri-dish, a known quantity of water containing a wetting agent 'Teepol' was added, and the surface of the colony gently rubbed with a glass rod to dislodge the spores. This process was repeated a number of times, as necessary, until the spores were all removed; the various washings were then combined, made up to a standard volume, and the concentration of spores in the final suspension estimated by three counts in a haemocytometer. Table 7 gives the summarized results of four experiments, the total number of spores per Petri dish being obtained by multiplying the figures in the table by 10^5.

TABLE 7. *Effect of TCNB on sporulation*

	Time in days							
	7		14		21		28	
Fungus	Control	TCNB	Control	TCNB	Control	TCNB	Control	TCNB
Botrytis cinerea	19	0	130	0	273	0	380	0
Fusarium caeruleum I	10	0	62	33	105	78	136	119
Fusarium caeruleum II	7	0	49	0	92	0	125	0
Trichoderma viride	166	0	253	2	945	21	·	·

TABLE 8. *Effect of TCNB on sporulation of colonies of* Botrytis cinerea *of different ages at time of application*

			Time in days after treatment					
			3		7		14	
Time of treatment	Diam. colony at treatment	No. spores at treatment	Control	TCNB	Control	TCNB	Control	TCNB
At inoculation	0	0	0	0	16	0	115	0
2 days after inoculation	3·6	0	9	0	76	0	171	0
4 days after inoculation	5·4	3	18	1	99	5	196	6
8 days after inoculation	8·5+	22	58	25	142	28	257	23
12 days after inoculation	8·5+	86	148	80	212	83	352	89

The effect, as usual, varies in degree with the fungus. With *Fusarium caeruleum* I spore production was only slightly reduced, in contrast to strain II, in which it was entirely suppressed even after 28 days. In this respect the latter approaches *Botrytis cinerea* which produced no spores after 70 days. *Trichoderma viride*, which is not markedly affected by TCNB in its linear growth, shows severe reduction of sporulation (*c.* 3% of control). When one compares the effect of TCNB on the sporulation of *Botrytis cinerea*, *Fusarium caeruleum* II and *Trichoderma viride* (Table 7) with that on linear growth of the same fungi (Table 2), it is seen that sporulation is more severely repressed by TCNB than is linear growth.

Table 8 shows how the sporulation of *Botrytis cinerea* cultures is affected by application of TCNB at various dates after inoculation (average of six experiments).

It is seen that once a colony is exposed to TCNB vapour, sporulation is checked irrespective of its age or of the number of spores already present.

The experiments of Table 8 were made with cultures which were inoculated at the centre of the Petri-dish. The results were the same when the spore inoculum was spread evenly over the surface of the plate.

Cultures of *B. cinerea* were allowed to grow in ordinary air for 12 days, by which time they were heavily sporing. Some were then exposed to TCNB vapour for 58 days, the remainder being untreated. Spore suspensions from the treated cultures, when spread upon potato agar, germinated and produced a sporing mycelial weft in a manner indistinguishable from control spores of the same age. Thus, after nearly 2 months, there was no evidence of fungicidal or even of damaging action by TCNB. The effect appears to be purely fungistatic.

COMPARISON OF EFFECTS OF TCNB AND PCNB

The method of preparing films of PCNB was the same as for TCNB and in all other respects the experimental technique was as before.

A strict comparison of the effect of the two compounds, molecule for molecule, would be difficult to carry out. Their solubilities in water are so low that they have not been measured for either. Both are somewhat volatile, TCNB more so than PCNB, as is shown in Table 9, for which the writer is indebted to Messrs Bayer Agriculture Ltd.

TABLE 9. *Weight of PCNB and TCNB in mg. required to saturate 1 c.m. of air*

Temp. (° C.)	PCNB	TCNB
10	0·27	1·32
20	0·97	4·67
25	1·8	8·34

TABLE 10. *Effect of TCNB and PCNB on linear growth*

			Time in days					
			6			15		
Fungus	Medium	Temp. (° C.)	Control	TCNB	PCNB	Control	TCNB	PCNB
Botrytis cinerea	Potato	20	7·2	+	1·7	8·5+	0·7	4·3
Fusarium caeruleum I	Potato dextrose	25	6·7	4·3	5·9	8·5+	6·4	7·7
Fusarium caeruleum II	Potato dextrose	25	6·3	1·7	3·4	8·5+	3·8	6·2
Rhizoctonia solani	Potato dextrose	25	8·5+	3·5	3·7	8·5+	6·0	5·6
Trichoderma viride	Malt	25	8·5+	5·3	3·8	8·5+	8·5+	8·5+

The vapour pressure of TCNB is thus 4–5 times that of PCNB at the same temperature. As all the experiments were carried out under conditions where the vapour of these compounds could reach saturation the results are always weighted on the side of TCNB in so far as higher concentration in the air environment is concerned.

Table 10 gives the results of four experiments on the effect of 0·1 g. TCNB and PCNB on linear growth.

Botrytis cinerea in particular and the two strains of *Fusarium caeruleum* were more affected by TCNB than by PCNB; *Rhizoctonia solani* was about equally affected by both and *Trichoderma viride* was distinctly more sensitive to PCNB. *Pythium deBaryanum* was also included in the experiment but neither substance reduced its growth. The qualitative effects already described for TCNB (p. 454) were repeated with PCNB and to much the same degree.

Table 11 gives a comparison of the two substances in regard to sporulation, the numbers given in the table being relative and on the same scale as in Tables 7 and 8.

TABLE 11. *Effect of TCNB and PCNB on sporulation*

	Time in days								
	7			14			21		
Fungus	Control	TCNB	PCNB	Control	TCNB	PCNB	Control	TCNB	PCNB
Botrytis cinerea	21	0	0	121	0	0	294	0	0
Fusarium caeruleum I	15	0	3	69	35	50	128	89	103
Fusarium caeruleum II	7	0	0	49	0	0	92	0	0
Trichoderma viride	151	0	0	227	1	0	895	24	5

The effect of PCNB on sporulation ran very close to that of TCNB. *Fusarium caeruleum* I was slightly more repressed by TCNB. *Trichoderma viride* behaved conversely and in accordance with the results on linear growth shown in Table 10. The sporulation of *Botrytis cinerea* and *Fusarium caeruleum* II was completely suppressed, so that no statement as to the relative effectiveness of the two substances on these two fungi can be made.

I wish to thank Professor W. Brown, F.R.S., for initiating and supervising this work and for preparing the text for publication.

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THE FUNGICIDAL CONTROL OF LETTUCE DOWNY MILDEW, CAUSED BY *BREMIA LACTUCAE*

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(With Plate 14)

Protection experiments carried out in the greenhouse showed that preparations of thiram, zineb, ferbam and ziram were superior to cuprous oxide and copper oxychloride preparations, in controlling downy mildew of lettuce and in lack of phytotoxic effect.

Tests made in winter on lettuce seedlings grown in Dutch lights, showed that *Bremia lactucae* attack reduced the stand of seedlings, diminished their size and weight, and, when the crop was planted out into the open ground, reduced survival in the field and slightly delayed the date of reaching maturity. The best overall results in protecting foliage against downy mildew were given by thiram and zineb preparations.

Introduction

Investigations on fungal diseases of the cultivated lettuce at the Imperial College Field Station, Slough, have largely been on those caused by *Botrytis cinerea* and *Rhizoctonia solani*, and on their control by chloronitrobenzene derivatives. These fungicides have proved ineffective against downy mildew. In 1939–41, Wild (1947) investigated the mildew problem and suggested the use of 50% cuprous oxide and excess lime Bordeaux (2 : 10 : 40). These gave moderate control but caused some damage. The present work examines the field performance of certain of the newer organic fungicides against lettuce downy mildew.

Mildew appears in apparently haphazard fashion in seedling lettuce overwintering in cold frames. It was extremely severe in frames on various market gardens in the Thames Valley in the winter of 1947–8, but there was practically none in the two following winters, and therefore when the problem was taken up in the autumn of 1950, it was necessary to induce an artificial build-up of infection.

The technique of raising spore material of *Bremia lactucae* is described by Powlesland (1954). In 1951–2 mildew was strongly established at an early stage and fungicide trials were made under conditions of high mildew infestation in the control frames.

Eighteen preparations* were tested, including weak Bordeaux mixture (2 : 2 : 40) and three other copper fungicides; two quinone derivatives and four dithio-carbamic acid derivatives. Cuprocide (90% Cu_2O) was generally used in the tests as a standard of comparison, Wild (1947) having found it the most promising of the substances he tested. Several of the materials used in the present work are specified more fully below.

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* The authors are indebted to Mr G. G. Taylor, formerly of the Fungicides and Insecticides Research Co-ordination Service, for supplying most of these preparations, and for helpful advice concerning their use.

GREENHOUSE EXPERIMENTS

Lettuce seedlings used for protective experiments in the greenhouse were grown in tins in batches of twenty. The expanded cotyledons were sprayed with the fungicides at the rate of approximately 1 ml. per tin. After the spray deposit had dried the plants were inoculated with a suspension of *B. lactucae* spores. The tins were kept 24 hr. in a moist chamber, then placed in a cold frame. A second wet period of 1–2 days, about 10 days after inoculation, was given to promote sporulation and then the percentage of plants infected was recorded. For each dose of fungicide, five or six tins, totalling 100–120 seedlings, were used. The fungicides were as a rule applied at concentrations of 0·25, 1, 4 and 16 g./l. of spray.

Copper-containing materials caused a spotting of the leaves: thiram and ziram induced leaf paling and curling and, exceptionally, a desiccation.

TABLE 1. *Comparison of protective efficiency of thiram and Cuprocide*

Fungicide	Dose (g./l.)	Percentage of plants infected: Exp. 1	Percentage of plants infected: Exp. 2
Thiram	4	4·6	3·4
	1	12·3	9·4
	1/4	63	34·3
	1/16	.	83
Cuprocide	4	13·7	19·5
	1	43	26
	1/4	79	54
	1/16	97	73
Control	.	99·4	100

A mixture of glyoxalidines gave no obvious control of mildew except at a dosage that was very damaging to the plants. Malachite green at the concentrations used gave moderate control without any phytocidal damage, but it was not considered promising because it was readily washed off. Bioquin (copper 8-quinolinate) gave no damage at any concentration used but failed even at the very high dose of 64 g./l. to give complete control.

Bordeaux mixture (2 : 2 : 40) and two proprietary copper preparations, Coppesan (50% $CuOCl_2$) and Cuprocide (90% Cu_2O) often caused damage at doses which were effective against downy mildew.

The superiority of thiram to Cuprocide is illustrated in Table 1 which gives the summarized results of two experiments.

A trace of damage was shown only by the highest dosage of thiram in Exp. 2; in the Cuprocide series some damage occurred at the highest dosage in Exp. 1 and more pronounced damage at the two highest dosages in Exp. 2. The degree of protection afforded by thiram at concentrations of 4 and 1 g./l. considerably exceeded that given by Cuprocide at the same concentrations.

Preparations of three derivatives of dithiocarbamic acid, zineb, thiram and ferbam, at concentrations of 4 g./l. gave similar results, as shown by Table 2.

TABLE 2. *Comparison of protective efficiency of three derivatives of dithiocarbamic acid*

	Percentage of plants infected			
Fungicide	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Zineb	2·9	0·9	0	0
Thiram	3·4	4·1	0	0
Ferbam	6·4	1·0	0·9	1·8

TABLE 3. *Lessening of protective action of fungicidal deposits with lapse of time*

No. of days between deposition of fungicide and inoculation	Percentage of plants infected		
	Thiram	Zineb	Control
0	0	0	100
2	0	0·8	100
5	1·5	0·8	90
9	5·7	3·2	97
12	7·1	17	97

The quinone derivatives, Spergon (tetrachlorobenzoquinone) and Phygon (dichloronaphthaquinone), were less fungicidal, at safe dosages, than any of the thiocarbamate preparations.

The greenhouse tests, made at various times of the year, ranged the preparations in the following order of diminishing value for mildew control:

$$\text{Zineb} = \text{thiram} > \text{ferbam} > \text{ziram} > \text{Phygon} > \text{Spergon}.$$

The first four materials were therefore mainly used for further trials.

Table 3 shows the loss of protectant effect with lapse of time, using thiram and zineb, both applied at 4 g./l.

The experiments so far described were all carried out in a greenhouse or cold frames and the leaves remained dry after inoculation. In the control of mildew in seedling lettuce grown in Dutch lights, the question of rain-resistance of the fungicide does not arise, as the plants are covered until within a few days of the date of transplanting into open ground. Nevertheless, an investigation was made on the effect of rain on a few of the more promising compounds. The materials used were thiram, zineb, ferbam and ziram, each applied to batches of twenty-four tins at a concentration of 4 g./l. and at the rate of 1 ml. per tin. The fungicidal deposit was allowed to dry and the plants then exposed to varying amounts of water, viz. batches of six tins (= *c.* 120 plants) being syringed with water at the rate of 1 ml. to each tin;

other batches at the rate of 4 ml. per tin; and others again placed outside in a heavy rain shower for 30 min. All were then sprayed equally with a suspension of *Bremia* spores. The results are given in Table 4.

TABLE 4. *Percentage of plants infected after various washing treatments*

Amount water applied	Thiram	Zineb	Ferbam	Ziram	Control
None	1·5	0	13	13	97·5
1 ml./tin	3·5	1·4	28	50	.
4 ml./tin	14·5	4·6	73	64	.
Shower, 30 min.	49·5	7	64	79	.

None of these substances, except zineb, showed tenacity even after a light washing.

FIELD EXPERIMENTS

These were carried out over 2 years (1950–2) in a range of about 120 Dutch lights. The experiments of the first season were preparatory and will be briefly summarized.

Inoculations were made in February (date of sowing, 20 October) by either of two methods: (1) applying 20 ml. of spore suspension with a de Vilbiss atomizer to each Dutch light; (2) inoculating a number of marked plants in each frame by hand. After inoculation, the frames were closed overnight after which some were kept closed and others ventilated.

As a result of these inoculations mildew appeared early in March. The frames that had been sprayed showed a widespread even infection ranging from 46 to 79%, and only slightly greater (77%) in frames that had been kept closed throughout than in those that had been ventilated (58%). In the frames where individual plants had been inoculated, spread to neighbouring plants was very slow, and at planting-out time (April) there were only 4% of infected plants in the well-ventilated frames and 19% in the non-ventilated.

Though a reasonably high percentage of infected plants was obtained by atomizing the inoculum, only a small proportion of the foliage was infected. The frames in which mildew had been established showed no inferiority either in size or number of plants, and when randomized successional plantings were made in the open ground, the infected plants gave equal survival with the controls. They matured at the same time and were of the same average weight. These results were obtained in a season when mildew did not spread in the field and when the plants were of the same size at transplanting.

Experiments with fungicides could not be included in the main (October) sowing, but after the frames had been cleared in early spring, they were resown. By sloping the frames to the north and by lime-washing the lights, temperatures were kept sufficiently low and humidity sufficiently high to allow heavy mildew attack of the unprotected plants. Three sowings were made over March–April, and about a fortnight after each sowing replicate tests of the more promising fungicides were made, as already described. The results of these experiments are summarized in Table 5.

Thiram and ziram at the highest dosages used, viz. 16 g./l. showed some phytotoxicity; otherwise negligible damage was caused by the organic compounds. The damage by Cuprocide was greater throughout, but less than in winter experiments with seedlings in pots. This accords with Wild's (1947) statement that the phytocidal action of Cuprocide is accentuated by cold weather.

In general the results of these preliminary protective experiments on a field scale corroborated those of the greenhouse tests.

TABLE 5. *Percentage of plants infected in frames with or without protectants*

	Thiram		Zineb		Ziram		Cuprocide		Control
	g./l.	Infn.	g./l.	Infn.	g./l.	Infn.	g./l.	Infn.	Infn.
Exp. 1	16	0	4	0	16	0	1	14·4	59
2	.	.	4	0·4	4	2·8	1	10	50
	.	.	1	0·2	.	.	.	.	.
3	4	0·5	.	.	.	.	1	7·0	71
	1	3·5	.	.	.	.	.	.	.

In the winter months of 1951–2 a much earlier and therefore more severe attack was established, thus providing a more rigorous test of the fungicides. A batch of thirty frames was sown on 11 October, and twenty-five were partitioned into half-frames. Each half-frame was protected by one of the three protectants used—thiram, zineb or ziram—while the other half was left unprotected. To ensure wetting of all leaf surfaces 25 ml. per half-frame was applied. The day after the application of the fungicides, all frames were sprayed with a suspension of *Bremia* spores, and the usual incubation followed. Randomized among this series were five frames which were not sprayed with either fungicide or *Bremia* spores, to give a measure of the natural spread of mildew within the range of frames. All the protective treatments were replicated 5 times, there being two dosages of thiram and zineb (see Table 6).

The dates of applying the fungicides and the state of growth of the plants at the time are shown in the following scheme:

Date of spray applications	No. of rough leaves on seedlings
7 Nov.	0
23 Nov.	2
17 Dec.	3
7 Jan.	4
22 Jan.	5

As a precaution against *Rhizoctonia* and *Botrytis* attack all the frames were dusted with a preparation containing 5% tetrachloronitrobenzene on 30 January and again at the time when the first batches were transplanted to the open ground (22 February).

By the third week of November downy mildew attack was showing on 40–60% of the plants in the unprotected half-frames. By the end of December it had also appeared in the frames which had not been sprayed with *Bremia* spores. On 9 January all the half-frames were sampled, fifty plants being taken at random from each. These were sorted into various categories and gave the average results set out in Table 6.

All five treatments gave substantial control of the disease and none caused any damage. The percentage of plants that retained their cotyledons was 96–99 in the protected series; 4–8 in the unprotected. The dropping of cotyledons by the unprotected plants can probably be ascribed to mildew attack.

TABLE 6. *Effect of protective treatments on mildew*

	Percentage protected plants with				Percentage unprotected plants with			
Fungicide (g./l.)	Sporing lesions	Dead mildew lesions	Cotyledons disappeared	Cotyledons healthy	Sporing lesions	Dead mildew lesions	Cotyledons disappeared	Cotyledon healthy
Thiram (16)	0·4	0·8	0	98·8	47·2	28·4	17·6	8·4
(4)	0·8	3·2	0	96	44·4	24·4	26·4	4·8
Zineb (4)	0·4	2·0	0	97·6	49·2	24·8	20·4	5·6
(1)	2·4	1·6	0	96·0	45·2	26·0	24·4	4·4
Ziram (16)	0	1·2	0	98·8	44·8	30·4	19·6	5·2

The weights (wet) of the fifty plants from each half-frame were determined on 9 January and further samples of fifty were weighed at the time of transplanting. The average weights of plants protected by the various fungicides were very similar; the overall averages were as follows:

	Protected g.	Unprotected g.
9 Jan.	30·0	19·8
28 Feb.	89·3	67·2

For each protectant, the differences in plant weight on 9 January were all significant at $P = 0{\cdot}01$; on 28 February the differences were significant or barely significant at the $P = 0{\cdot}05$ level.

The superiority of the protected plants was still pronounced at the time the frames were cleared for transplanting. The differences between the five fungicidal treatments were negligible and the records (%) are combined in the following:

	Protected	Unprotected
% plants with mildew lesions	0·53	50·2
% plants with *Botrytis* lesions	1·3	23·5
% plants apparently clean	98·2	26·3

Both mildew and *Botrytis* attack were much reduced by all the treatments. Control of the latter may have been effected either directly or by control of mildew which predisposes the plants to *Botrytis* invasion.

The record of final stand in the half-frames and of the survival of the plants in the field up to maturity is contained in Table 7. Data for protected plants, which are significantly greater ($P=0{\cdot}05$) than the corresponding ones for the controls, are in black.

All the treatments increased the number of plants in the frames, though only in two cases did the difference reach significance. Both treatments with thiram and that with ziram gave improved survival in the field. Somewhat unexpectedly the treatments with zineb, which gave good results in the frames, showed no beneficial effect in the field. The beneficial effect of protectants is illustrated in Pl. 14, Figs. 1 and 2.

TABLE 7. *Effect of protectants upon number of plants at date of transplanting and upon survival to maturity*

	Protected		Unprotected	
	Stand in frames	Percentage survival in field	Stand in frames	Percentage survival in field
Thiram (16)	**330**	**86·4**	241	68·4
(4)	331	84	262	71·2
Zineb (4)	**366**	67·6	234	74
(1)	299	71·6	249	66
Ziram (16)	332	**88·8**	277	72

A second transplanting carried out 1 week later gave substantially the same results on survival as those shown in Table 7, and again the performance of zineb in the field was disappointing.

The effect of mildew on the date of maturity was also determined for both sets of transplantings. Ten comparisons were available between protected and unprotected batches: in nine of these the plants that had been protected matured before the unprotected controls, and in six of these the difference was significant at the 0·05 level. These results were obtained in April–May 1952, when the weather was unusually warm so that the plants hearted up very quickly. If the weather had been cooler, the initial check caused by mildew would probably have resulted in a greater check to maturing. The average weights of mature plants were equal in the protected and the unprotected plants.

The overall conclusion from this experiment is that mildew, of the intensity recorded above (Table 6), reduced the number of seedlings available for transplanting, diminished their size and weight, curtailed their survival in the field and somewhat delayed their date of maturity.

The five frames in which the plants were neither protected nor sprayed with *Bremia* spores, showed an average of 54% natural infection at transplanting. The disease was late in developing on these plants and no effect on their growth or numbers was established. Both in the frames and in the field their performance was indistinguishable from that of the protected plants.

A direct comparison of thiram, zineb, ziram and ferbam, at the same dosage, viz. 4 g./l., was made in two experiments each made on thirty-two half-frames. One series (Exp. 1 in Table 8) was sited on a light sandy soil and the other on a fertile loam. These experiments were run concurrently with the one described above, and the details of sprayings, management, sampling, etc., followed the same plan. The essential results of these two experiments are contained in Table 8. The figures for intensity of mildew infestation refer to the date of transplanting.

TABLE 8. *Effect of protectants on downy mildew infection and on growth and survival in the field of lettuce seedlings*

	Protected				Unprotected			
	Percentage mildew	Wt.*	Final stand	Survival	Percentage mildew	Wt.	Final stand	Survival
				Experiment 1				
Thiram	5·1	88	334	82·5	62	49	251	73
Zineb	0·7	95	330	77·5	49	57	249	62·5
Ziram	8·5	74	329	75·5	58	45	253	57·5
Ferbam	18·3	80	350	84·5	57	46	282	60
				Experiment 2				
Thiram	14·7	81	414	—†	92	45	276	—
Zineb	5·4	89	417	—	84	42	302	—
Ziram	28	79	345	—	90	44	321	—
Ferbam	45	90	387	—	90	50	316	—

* Weight, in grams, of fifty plants.
† This series not transplanted.

Plants treated with ziram and ferbam, especially the latter, showed inferior mildew control at transplanting. In an earlier sampling these fungicides had given good results, and markedly delayed the onset of mildew. It is probably for this reason that their final effect on the crop was not inferior to that of thiram and zineb. The various effects shown in Table 8 follow the general pattern recorded for the preceding experiment.

In Exp. 2 of Table 8, where the plants were on fertile loam, there was a greater development of mildew than in Exp. 1. These plants grew more vigorously but the greater growth is not reflected in the figures of plant weight, as they were sampled two weeks earlier.

Finally a batch of thirty frames was set up on land known to be contaminated with *Rhizoctonia solani*, and sown with lettuce in the usual manner. These were used for an exploratory experiment to test whether any of the organic fungicides which had given good control of mildew would likewise exercise a useful control of the *Rhizoctonia–Botrytis* disease complex. These frames were somewhat segregated from the remainder and escaped natural contamination with mildew. Eight fungicidal treatments were used, and each of these was applied five times during the winter season.

POWLESLAND AND BROWN—*Fungicidal control of lettuce downy mildew*

In this experiment there was considerable variation between replicates, an outcome of the well-known patchy distribution of *R. solani* in soil. The broad result, however, was that all the treatments, viz. thiram at 16 and 4 g./l., zineb at 4 and 1 g., ziram at 16 g., and ferbam at 4 g. increased the average stand of plants at the time of transplanting, so that some measure of control of *Rhizoctonia–Botrytis* was suggested. Only one of the treatments however, viz. thiram at 16 g./l., had any pronounced effect on the freedom of the seedlings from lesions. The percentage of plants with lesions was 22·5 for this treatment as against 65–90% for all the other treatments and the controls. It would therefore be of interest to make a careful comparison of thiram and tetrachloronitrobenzene with regard to the control of damping-off and *Botrytis* disease of lettuce.

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EXPLANATION OF PLATE 14

Fig. 1. Left, protected with thiram 16 g./l. Right, unprotected.
Fig. 2. Left, protected with ziram 16 g./l. Right, unprotected.

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HEAT-THERAPY OF VIRUS-INFECTED PLANTS

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(With Plate 15)

Virus-free plants were produced from parents systemically infected with the following five viruses: tomato bushy stunt, carnation ring spot, cucumber mosaic, tomato aspermy and *Abutilon* variegation. The leaves formed while the infected plants were kept at 36° C. were free from symptoms, and test plants inoculated from these remained uninfected. When cuttings were taken from the infected plants at the end of the treatment most grew into healthy plants. The treated plants themselves usually developed symptoms after varying lengths of time at 20° C., but some that before treatment were infected with tomato aspermy, cucumber mosaic or Abutilon variegation viruses, remained permanently healthy.

The same method failed to cure plants infected with tomato spotted wilt, potato virus *X* and tobacco mosaic virus, although it decreased their virus content. Heat-therapy seems not to be correlated with the thermal inactivation end point of the virus *in vitro*.

After showing that peach trees were cured from yellows when kept at 35° C. for 2 weeks or more, Kunkel (1936, 1941) also found peach and periwinkle plants could be freed from certain other viruses by growing them continuously at high temperatures. None of the viruses studied by Kunkel has been transmitted mechanically and nothing is known about their stability *in vitro*, but his results were generally assumed to indicate that the viruses are heat-labile and have low thermal inactivation points. The demonstration that some viruses with high thermal inactivation points fail to cause lesions when recently inoculated plants are placed at 36° C., suggested that this assumption was not necessarily justified (Kassanis, 1952). The susceptibility of plants to infection by several mechanically transmissible viruses was increased by keeping plants at 36° C. before they were inoculated, but the effect of exposing plants to 36° C. after they were inoculated varied with different viruses and was not correlated with the thermal inactivation point of the virus *in vitro*. Tomato spotted wilt virus, with a thermal inactivation point of 45° C., and tobacco mosaic virus, with one of over 90° C., both formed lesions in inoculated plants kept at 36° C., whereas tomato bushy stunt and tobacco necrosis viruses, with thermal inactivation points of over 80° C., and cucumber mosaic virus, with one of 70° C., did not. This fact, together with the observation that the virus content of leaves of *Datura stramonium* systemically infected with tomato bushy stunt virus decreased with increasing time the plants were kept at 36° C. (Kassanis, 1952), suggested that heat-therapy might be effective in freeing infected plants from some heat-stable viruses. The results described in this paper show that this is so.

METHODS

The incubator is a miniature glasshouse, with a wooden frame 75 cm. wide, 105 cm. long and 90 cm. high, and walls and sloping roof of glass. The plants in pots stand on a slatted wooden floor, raised 4 in. from the bottom. There are four steel-jacketed heater units, each of 230 W., which are fixed on metal feet and are 5 in. from the slatted floor, from which they are separated by two asbestos baffle boards that protect the plants from direct heat. Ventilation is provided by a row of seven 1 in. holes on each side, opening to the outside from the space containing the heaters, and also by a similar row of holes along the central ridge of the roof. A small fan mounted vertically in the roof ridge stirs the air. The heaters are controlled by a Sunvic thermostat and a temperature of 36 ± 1° C. was obtained.

Twelve 5-in. pots, standing in glass saucers containing water, can be accommodated in the incubator. The water in the saucers is replenished from a container kept in the incubator, so that the water used is at 36° C.

The plants, unless otherwise mentioned, were infected for at least 3 weeks and showed systemic symptoms when they were placed in the incubator. The plants were kept at 36° C. for 3–4 weeks, during which time they usually grew very little, although *Abutilon striatum* (Dicks.) and cucumber plants made substantial growth. A few lower leaves usually withered during the treatment, and the newly formed ones, especially those of tobacco, were darker green, had shorter main veins and were more crinkly, than leaves of plants at lower temperatures. Carnation plants were the most difficult to keep alive at high temperature.

More plants survived the treatment in the summer than in the autumn and spring, when additional hours of artificial light were necessary to keep them growing. This was provided by four strip lights (Philips MCFB/U 40 W., Natural) fixed on the outside of the roof of the incubator. In the winter, even with artificial light, the plants could not be kept in the incubator for more than 2 weeks. Well established plants stood the treatment better. Young plants or those recently potted usually died after a few days at 36° C.

When possible, cuttings were taken from the side shoots of treated plants, but with tobacco plants and with small tomato plants the main shoot was cut and rooted. The cuttings were rooted in sand, usually without a rooting hormone, but with both methods several cuttings perished before rooting.

EXPERIMENTAL

Table 1 summarizes the results of experiments in which heat-therapy produced virus-free plants from parents systemically infected with five viruses. All except *Abutilon* variegation are mechanically transmissible. New leaves produced by plants at 36° C. showed no symptoms (Pl. 15, fig. 1), and sometimes young leaves, which showed definite mottling when the plants were put in the incubator, became symptomless after some days at 36° C. This loss of symptoms was particularly striking with variegated *Abutilon striatum* (Dicks.) plants. At the end of their period in the incubator, some leaves from all surviving plants (except. *A. striatum*) were macerated with pestle and mortar and their juice inoculated to leaves of healthy test seedlings. These seedlings had their leaves dusted with 'Celite' before inoculation to aid infection by any virus particles present in the inoculum, but none of them ever became infected. Despite the failure to transmit anything by inoculation from the leaves of the treated plants, many of the plants were still not wholly freed from the viruses, for when they were kept under ordinary glasshouse conditions at about 20° C. they again developed symptoms. The time taken for this to happen varied with different plants and was longer the longer the plants had been kept at 36° C. All the parent plants infected with tomato bushy stunt or carnation ring spot viruses ultimately developed typical symptoms after a month or more at 20° C. The production of healthy plants from parents infected with these viruses depended on striking new plants from cuttings taken from the side shoots or main stems of the treated plants at the end of their stay at 36° C. Not all of the cuttings remained healthy. From the few that developed symptoms, viruses were then regularly transmitted to other plants. Repeated attempts over periods of 4–6 months to transmit any virus from those that remained healthy all failed, and it seems certain that they are virus-free. Some *A. striatum* cuttings have now been growing for a year without developing any variegation (Pl. 15, figs. 2 and 3), but they readily do so when grafted with scions from variegated plants. All the cuttings from treated plants that had cucumber mosaic virus and tomato aspermy remained

Table 1. *Inactivation of five viruses in all or part of the infected plants kept at 36° C.*

Virus	No. and species of treated plants	Length of heat treatment in days	The condition of treated plants after being returned to ordinary temperature*	No. of rooted cuttings which remained healthy
Tomato bushy stunt	12 *Datura stramonium*	23	Symptoms reappeared in 10 days	8/10
	10 *Datura stramonium*	32	Symptoms reappeared in 3–5 weeks	4/5
Carnation ring spot	11 carnation	24	Symptoms reappeared in 2–3 weeks	13/15
Cucumber mosaic	4 cucumber	32	Remained healthy	2/2
	12 *Datura stramonium*	27	Remained healthy	6/6
	10 cucumber 2 tobacco	23	One cucumber remained healthy, the rest developed symptoms at different times up to 2 months	10/10 2/2
Tomato aspermy	4 tobacco 4 tomato	21	Remained healthy	4/4 3/3
	6 tomato	26		4/4
Abutilon variegation	1 *Abutilon striatum*	28	Remained healthy	1/1
	4 *Abutilon striatum*	26	Symptoms reappeared at different times up to 1 month	2/4

* Infectivity tests were made on *Datura stramonium* for tomato bushy stunt virus; Sweet William plants for carnation ring spot virus; tobacco plants for cucumber mosaic and tomato aspermy viruses.

healthy and no virus has been transmitted from them. Many of the parent plants infected with these two also seem to have been freed completely from the virus, for they also failed to show symptoms when kept for 4–6 months and nothing could be transmitted from them to young test seedlings.

Kunkel (1936) also found that some of the plants he kept at high temperatures again developed symptoms after some time at lower temperatures. He attributed this to the fact that although the aerial parts of his plants were freed from the viruses, the roots were still infected. This could also explain my results, for although the pots were supplied with water at 36° C., evaporation of water from the soil and saucers lowered the soil temperature to 33 or 34° C. The development of symptoms in some of the cuttings, however, also suggests that virus can be erratically distributed in aerial parts, and that when most have been freed some tissues can still be infected.

The mechanism underlying the cure is unknown. It may be that a temperature of 36° C. directly inactivates these viruses, but they would not be denatured *in vitro* by this treatment. Perhaps it is more likely that the virus content of infected cells reflects an equilibrium between synthesis and degradation of virus particles and that synthesis fails at 36° C. Continuation of the normal degradation process would then lead to these viruses disappearing from infected tissues.

That heat-therapy may not depend on a direct effect of heat on virus particles is suggested by failure of treatment at 36° C. to free tomato plants from tomato spotted wilt virus, which has a thermal inactivation point of only 45° C. There are many reports of virus diseases the symptoms of which become less severe as the temperature is increased. Spotted wilt in tomato is an exception to this behaviour, for the symptoms are intensified by moving plants from 20 to 36° C. Within 3 days at 36° C., infected plants, that previously showed only leaf mottling and crinkling, became generally chlorotic and developed necrotic streaks on stems and leaves. Leaves started to shrivel and all plants died after a week or more. The enhanced symptoms did not reflect an increased multiplication of the virus. Indeed, virus multiplication was less at 36° C. than at 20° C., for juice from plants treated for a week produced many fewer lesions on tobacco than juice from control plants kept under ordinary glasshouse conditions. Plants that survived a week at 36° C., and cuttings from them, all developed typical symptoms when they were later kept at 20° C. No experiments were made with tomato spotted wilt virus in other hosts, so that it is unknown whether the enhancement of symptoms at 36° C. is a general phenomenon or whether infected plants of some other species would survive longer at 36° C. and perhaps be freed from this virus.

Experiments with other viruses with high thermal inactivation points showed that these also multiply less readily at 36° C. than at 20° C., but tissues systemically infected with these were not freed by prolonged exposure to 36° C.

Potato virus *X* multiplies poorly in inoculated leaves of tobacco or *Datura stramonium* plants put at 36° C. after inoculation, and spread of the virus to young leaves is much delayed. Eight days after inoculation, the leaves at 36° C. contained enough virus to infect test seedlings but too little to give a specific precipitate with the virus antiserum, whereas juice from control leaves at 20° C. precipitated up to a dilution of 1/256. When systemically infected potato, tomato and *D. stramonium* plants were kept at 36° C. for 23 days, their virus content, as shown by infectivity tests to tobacco seedlings, was much below that of plants kept at 20° C., but none of the cuttings taken from these plants gave healthy progeny.

Tobacco mosaic virus also multiplies much less readily in inoculated leaves at 36° C. than at 20° C., although it is less affected than potato virus *X*. Sap extracted from inoculated leaves 8 days after inoculation precipitated with virus antiserum at dilutions up to 1/8 compared with 1/2000 for sap from inoculated leaves on plants kept at 20° C. In systemically infected young leaves, multiplication of tobacco mosaic virus seems less affected by the high temperature, and sap from young leaves of plants at 36° C. gave a precipitation end point of 1/500 compared with that of 1/2000 for sap from comparable leaves of plants at 20° C.

It is clear that, although all these viruses multiply much less readily at 36° C. than at 20° C., and that heat-therapy can be applied to some viruses with high thermal inactivation points, it is not applicable to all, and not to some with low thermal inactivation points. Too few viruses whose shape and properties are known have yet been studied for any safe generalizations to be made. It may be significant, however, that three of these to which heat-therapy has been successfully applied, tomato bushy stunt, cucumber mosaic virus and carnation ring spot virus, have spherical particles and small temperature coefficients of thermal inactivation. Two of those already mentioned, with which heat-therapy has failed, potato virus *X* and tobacco mosaic virus, and two others with which preliminary results also suggest probable failures, potato virus *Y* and henbane mosaic virus, have rod-shaped particles and large temperature coefficients of thermal inactivation.

I am indebted to Dr J. W. Blencowe for his help in designing the incubator.

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EXPLANATION OF PLATE 15

Fig. 1. Cucumber plants freed from cucumber mosaic virus by heat. The two plants to the right are infected with different strains of cucumber mosaic virus, and were photographed 40 days after infection. The two plants on the left were placed at 36° C., 17 days after they were infected and photographed after being at 36° C. for 23 days.

Figs. 2 and 3. *Abutilon striatum* (Dicks.) plants of the same age. Fig. 2 raised from a cutting taken from variegated plant kept continuously at ordinary glasshouse conditions. Fig. 3 raised from cutting taken from variegated plant after 28 days at 36° C.

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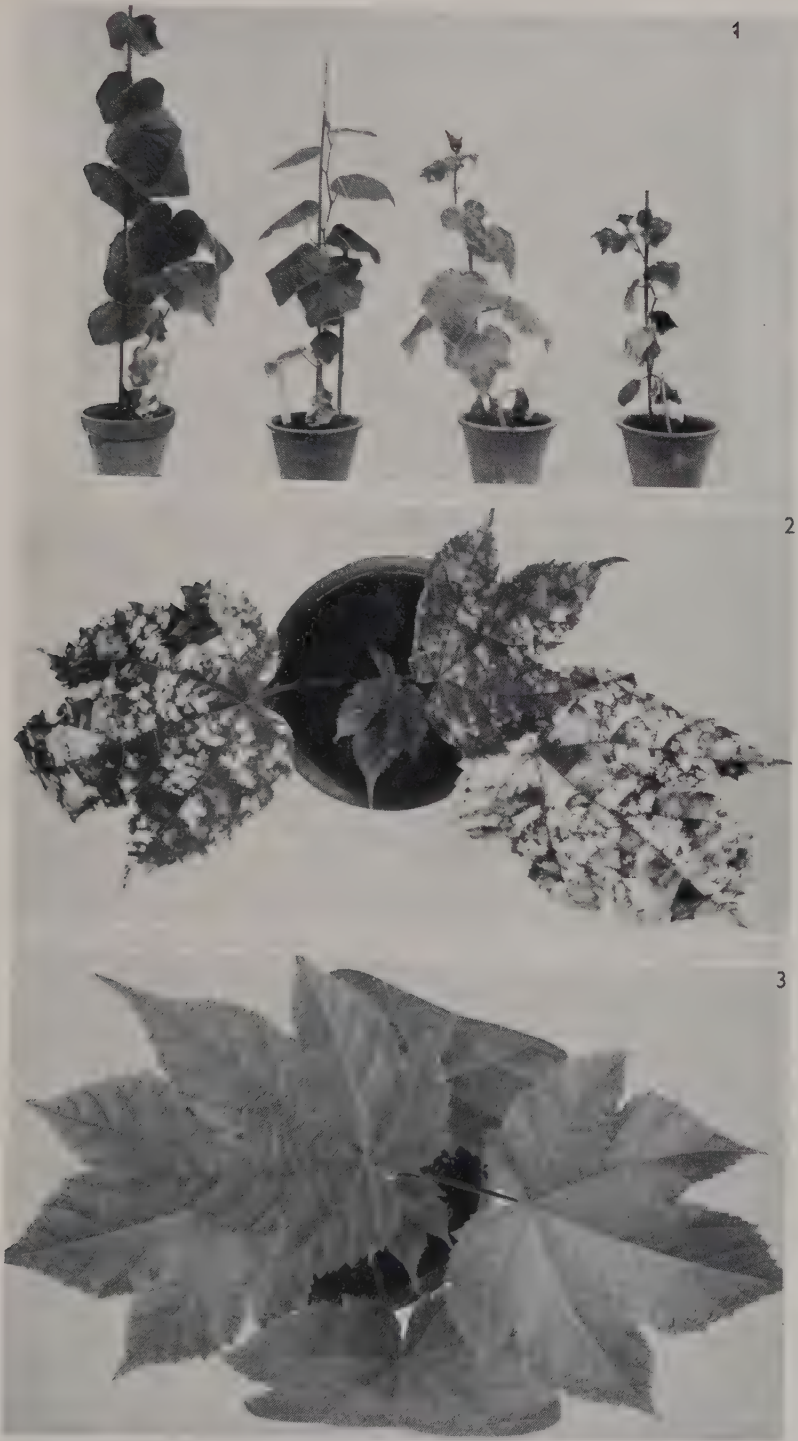

Kassanis—*Heat-therapy of virus-infected plants*

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THE ABSORPTION, TRANSLOCATION AND BREAKDOWN OF SCHRADAN APPLIED TO LEAVES, USING ^{32}P-LABELLED MATERIAL

I. EXPERIMENTAL TECHNIQUES

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The methods of propagation of plant material for experimental use, the procedure for preparing the radioactive solution, and its application to leaves either by dipping or spraying are described.

A description is given of the conditions under which the plants were kept, the procedure adopted at sampling involving leaching of the treated leaves, subdivision of the plant and preparation of samples for determination of 'schradan' and 'schradan equivalent' by liquid counting.

The methods of compiling results to determine the distribution and status of the applied schradan in or on the leaves at harvest, are outlined.

INTRODUCTION

Knowledge of systemic insecticides has developed rapidly in the last few years since such properties were demonstrated in certain organic compounds by Schrader (1947). Octa methyl pyrophosphoramide or schradan is the material on which probably most work has been done, and its insecticidal behaviour following absorption by roots and foliage have been described by many workers.

This series of papers summarizes investigations using the ^{32}P labelled schradan involving several thousand radio assays carried out between 1950 and 1953 on some of the factors associated with evaporation, absorption, translocation and breakdown following foliar application to apple stocks, broad and runner beans, chrysanthemums and *Coleus*.

Part I describes the general experimental technique and procedure adopted throughout the work.

Part II deals with the evaporation and absorption of the schradan from the leaf surface.

Part III with its translocation and breakdown within the plant.

1. PLANT MATERIAL

Methods of propagation

(i) Apple seedlings were raised from seed of the variety Dabinett, cold-treated and then sown in a heated glasshouse in January. The seedlings, in 3-in. pots, were used from June onwards. The height varied between 18 and 36 in. with an average of twenty expanded leaves per plant.

(ii) Apple stocks, Malling type VII. Stocks from the stool bed were potted into 6-in. pots in early winter. The pots, plunged in ashes, remained out of doors until late January, when they were brought into a heated glasshouse and cut back first to four or five basal buds, and later to the strongest bud, removing any lower buds. This gave an upright single stem. By varying the time of bringing the stocks into the heated glasshouse, suitable plants were made available over a longer period. Bud burst could be delayed by keeping the stocks in cold store, but prolonged cold treatment produced weak unsuitable growth.

(iii) Chrysanthemums. The variety Kathleen was propagated from cuttings in 3-in. pots. Plants were single-stemmed, 12–18 in. long when used, but the time taken to reach this stage varied according to the time of year.

(iv) *Coleus*. Two varieties were used, one rich in anthocyanins, and the other with these pigments lacking. They were propagated from cuttings throughout the year in 3-in. pots.

(v) Runner beans (*Phaseolus multiflorus*) were grown from seed in 3-in. pots and used when the first two leaves were well expanded.

(vi) Broad beans (*Vicia faba*) were also raised from seed in 3-in. pots and used from the 3–4 expanded leaf stage onwards throughout the year.

2. CHEMICAL MATERIALS

(*a*) *Radioactive schradan*. The radioactive material used for the whole series was, with one exception, supplied through the courtesy of Pest Control Ltd. The percentage of radioactive phosphorus present in the chemical form of schradan varied for each batch but was usually stated to be within the range 97–99·9 % but two samples used only in exploratory experiments were of 84 and 94 % purity respectively. The specific activity varied between 150 and 950 microcuries (μc.)^{32}P/g. schradan. The inactive impurities, varying between 2 and 4 %, consisted mainly of trisdimethylamino phosphine oxide. The one other sample used was supplied by Drs B. A. Kilby and J. G. Gardiner. This sample was very pure but the specific activity was low—about 50 μc./g. schradan.

(*b*) *Preparation of solution*. Solutions for application were prepared from the material as received, supplemented if necessary by the addition of pure non-radioactive schradan and made up to volume with water in a graduated flask. The schradan concentration of the solutions used varied between 0·005 and 1·0 % with an activity of up to 2·5 μc./ml. For some experiments a wetter (Estol H, soft soap, or Cellofas at 0·25 %) was included.

(*c*) *Purity of material*. Hartley, Heath, Hulme, Pound & Whittaker (1951) found that the distribution coefficient (at 25° C.) of pure schradan between chloroform and 1 N caustic soda is 24. Distribution coefficient checks were made on all the radioactive samples received, and satisfactory agreement with the value claimed obtained in each case.

3. APPLICATION OF MATERIAL TO PLANTS

Pretreatment of plants

Unless otherwise stated in the text, all plants were 'conditioned' by subjecting them to a pretreatment period of 24 hr. darkness.

(i) *Dipping technique.* The solution, which for this method always included a wetter, was placed in a Perspex cell, large enough to allow the leaf to be immersed flat. As each plant was brought from the dark, all the leaves on one side were given a quick immersion and the excess liquid allowed to drip back into the cell. The plant was kept on its side until these leaves were dry, after which leaves on another side of the stem were similarly dipped. This process was continued until all the required leaves had been treated. Allowing the plants to dry on their sides avoided contamination of the stem, but this procedure was both laborious and slow; thus it took nearly 2 hr. to treat seventy-two mid-section leaves on nine apple stocks.

(ii) *Spraying.* The most suitable spray gun was found to be an Aerograph Air Brush, used in conjunction with an Edwards compressor operating at 15 lb./sq.in. This gun produced a finely atomized spray which was fairly evenly distributed when the gun was held about 1 in. from the leaf surface. Contamination of the rest of the plant was prevented by using filter-paper shields.

(iii) *Gun performance and estimation of deposit on leaf surface.* To obtain quantitative information on the amount of solution deposited on the leaf surface the procedure for filling and emptying the gun was standardized and determinations were made of the amount deposited as a proportion of that leaving the gun. The procedure finally adopted was as follows.

The Air Brush was thoroughly cleaned, dried, the cup filled with the solution and sprayed until empty on to waste filter paper. A known volume of the same solution was then run into the cup from a 1 ml. burette, and this sprayed on to the leaf surfaces using a 6 × 6 in. filter shield to back the leaves. In general both upper and lower leaf surfaces were sprayed, the exceptions being in experiments designed to obtain information on the relative absorptive capacity of upper and lower leaf surfaces. Spraying was continued until the gun was empty. If more solution was required to complete the spraying on the same plant a further known volume of solution was added and the procedure repeated. The same spray shield was used for all necessary sprayings on one plant, and after completion of spraying the shield was placed in a 400 ml. beaker, digested with conc. HNO_3 and radio-assayed (see p. 479). As some of the smaller droplets in the spray jet might not impact, experiments were carried out to find if the ratio of the volume impacting to the volume sprayed was a constant for different leaf and inert surfaces. Details of these experiments are given in an Appendix. The mean amount deposited on the leaves was 89% of that placed in the gun less the amount recovered on the spray shield.

(iv) *Subdivision of plant.* In some experiments arbitrary subdivisions of each plant were made for both application of stock solution and for harvesting. The aerial portion of each plant was envisaged as three sections with approximately equal numbers of leaves. One of these sections only was treated, and the separate harvesting of these pre-selected parts enabled information on the behaviour of the applied material to be obtained.

4. POST-SPRAYING TREATMENT

No facilities were available for controlled environmental conditions throughout the year. Plants were therefore kept under the conditions most favourable to growth. For late spring and summer experiments, an unheated glasshouse was used, in which adequate ventilation could be provided. From late autumn to early spring a special laboratory was used, having large windows on the south and west sides, and roof lights. The temperature of this room was maintained at about 75° F. To study the effect of light a box 150 × 90 × 90 cm. was constructed which could be illuminated by two adjustable 40-W. Mazda fluorescent daylight tubes (type MCF/U), placed normally about 6 in. above the growing point. The temperature within the box was similar to that in the laboratory, but air circulation was probably more restricted. For one series of experiments on the relative effects of temperature and light, four separate glass-walled compartments 120 × 75 × 75 cm. high were used in the glasshouse. Two of these were completely darkened and two were unscreened. In one of the dark and one of the light chambers the temperature was kept at 80° F. using a Sunvic control and a 500-W. heater placed on the floor of the chamber. To prevent direct radiation on the plants they were placed on a sand-covered asbestos tray mounted above the heater. Temperature distribution through the compartment was found to be uniform. The temperatures in the other two chambers followed that of the glasshouse which either by ventilating or supplementary heating was maintained at about 50–60° F.

Plants were watered by standing the pots in earthernware saucers of water.

To obtain information on the effect of rapid air changes on evaporational rates from glass slides and leaf surfaces, the following circuit was designed. Cylindrical glass towers 35 cm. high and 11 cm. diameter were sealed with glass end-plates, each having a central hole through which a glass tube was fitted. An Edwards compressor was used to draw air from the tower through the upper outlet tube. The air-flow rate was controlled by means of a T-piece screw clip assembly, and measured by passage through an air-flow meter, previously calibrated. Before entering the tower through the inlet in the base plate the air was dried by passage over $CaCl_2$.

In some experiments for assessing evaporational losses sintered glass gas bubblers containing water or chloroform were placed between the outlet tube from the tower and the flow meter to obtain a check on the evaporated material. At the end of the experiment the solutions in the bubblers were directly radioassayed.

Air-flow rates varying between 28 and 132 l/hr. were used in the tests, and where necessary two or more towers could be operated under similar conditions but varying air-flow rates.

The slides or foliage under test were placed where required in the tower.

5. HARVESTING

(i) *Leaching methods and materials.* The treated leaves were picked and then leached to remove any unabsorbed material on the leaf surface (Bennett & Thomas, 1953). The composition of the aqueous leaching solution which was contained in a series of staining jars was 0·001 % non radioactive schradan in which 0·25 % Estol H wetter was incorporated for some experiments. The procedure was as follows: the first leaf was submerged and gently agitated for 30 sec. in each of jars 1, 2 and 3. The second leaf received similar treatment in jars 2, 3 and 4 and the third in 3, 4 and 5. When all the treated leaves from one plant had been leached, the solutions from the jars were transferred to a graduated flask (250 ml.–2 l.) and the jars rinsed with fresh dipping solution into the flask, which was finally made up to volume with water. The efficiency of this procedure was confirmed by the failure of further leaching (up to 3 min.) to remove any more material from the leaves. It was therefore assumed that schradan present in leaf samples after this treatment had been absorbed.

If it was anticipated that the total leachable material would be small, a number of leaves were successively dipped in the same jars, to keep the final volume low and so obtain a measurable radioactive count.

(ii) *Subdivision of plant.* After leaching the treated leaves, the rest of the plant was harvested in pre-selected sections which were kept separate. When the roots were required for assay they were first washed free of soil by a jet of water (Bould, Nicholas & Thomas 1953).

6. RADIOASSAY

All radioassays were made by solution counting using the M6 tube (Veall, 1948) enclosed in a lead castle. The scaler was Model 22 supplied by Panax Equipment Ltd. Radioactive decay of the ^{32}P and any normal scaler fluctuations were allowed for by assaying the parent stock solution after every three or four experimental solutions.

To assess the ^{32}P content only of the section irrespective of its chemical state, the whole section was digested with conc. HNO_3 in a 400 ml. beaker until a clear solution was obtained. This solution was heated to drive off excess acid, made up to volume with water in a graduated flask and radioassayed.

The chemical state of the ^{32}P was determined by extraction and distribution methods described below.

(i) *Extraction from plant material and determination of extraction efficiency.* At harvest the particular section (after leaching where necessary) was cut into small pieces and either: (*a*) placed directly in a round-bottomed flask and a known volume of water containing a trace (0·001 %) non radioactive schradan added, or (*b*) placed in a boiling tube which was then stoppered and immersed in CO_2-acetone for 30 min. and stored at −20° C., to prevent further enzyme activity which might increase the decomposition of the schradan. Freezing also ruptured the cells, thus facilitating later extraction. The tube contents were transferred to a round-bottomed flask and a known volume of 0·001 % non radioactive schradan added, as in (*a*). This method of storage permitted the harvesting of a large number of samples at one time, and their maintenance in a fresh condition.

Usually 125 ml. of solution, less the water content of the sample, was placed in the flask with the plant tissue, but this volume was increased up to 500 ml. according to the bulk of plant tissue in the sample. The contents of the flask were refluxed for 2 hr. When cool, an aliquot, usually about 4/5 of the total volume, was filtered off into a graduated flask and the residue, with the filter paper, were placed in a 400 ml. beaker, digested with conc. HNO_3 and finally made up to volume in a graduated flask. The filtrate and residue solutions were then radioassayed for total ^{32}P content. From the initial volume of extracting solution and the volume of the filtrate, the amount of ^{32}P which should be in the filtrate could be calculated and compared with the observed figure, thus obtaining a measure of extraction efficiency.

(ii) *Estimation of schradan decomposition.* The extent of decomposition of the schradan in the filtrate obtained by extraction was determined by the general procedure of Hartley & Heath (1950) and Hartley *et al.* (1951). The solution was made $N/5$ by the addition of solid NaOH, refluxed for 15 min. to decompose any biochemically derived compounds, chilled and made $1N$ or $2N$ with solid NaOH. An aliquot of this solution was withdrawn and immediately partitioned with an equal volume of redistilled chloroform. An aliquot of the alkaline layer was pipetted off and most of the chloroform phase run from the separating funnel and a known volume quickly evaporated to dryness at 55–60° C. This residue was taken up in water and made up to volume in a graduated flask. Comparison of counts on the alkaline layer of the partition (corrected for density effect on observed count) and on the aqueous solution obtained from the chloroform layer indicated the extent of schradan degradation. In later stages of this work direct counting of the chloroform solution using an empirically determined correction factor to calculate the corresponding aqueous count was used. This factor was determined on a solution of pure radioactive schradan in redistilled chloroform. An aliquot was taken to dryness at 55–60° C. and the residue then made up to known volume in water. Observed counts on this solution and with the chloroform solution permitted the evaluation of this $CHCL_3$/water ratio.

7. PRECISION OF RADIOASSAY AND COMPILATION OF RESULTS

The precision of radioassay varied considerably depending on the level of activity present in the solution. In highly active solutions a precision of 98–99 % was achieved, but the majority of solutions were counted to within a variation of 5 %. With a few of the solutions in which the observed count was only about 50 % above background, variations of up to 20% had to be accepted. The procedure recommended by Loevinger & Berman (1951) was followed to decide the optimum ratio of sample and background counts.

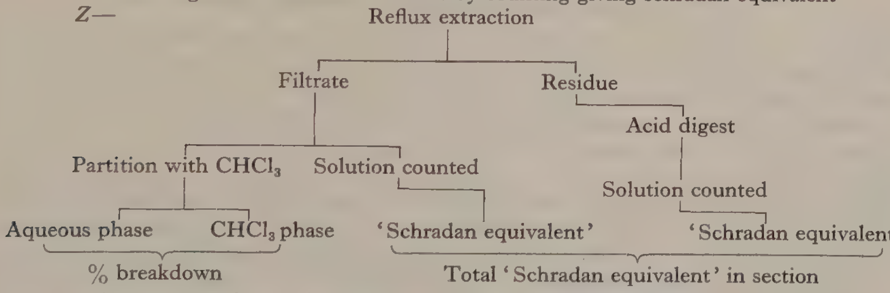
Section 6 shows that radioassay data were obtained on the total ^{32}P concentrations in the solutions counted. The absolute efficiency of the M6 tube used for counting need not be known, but for these calculations it is assumed to be such that a solution having a concentration of 1 μc. ^{32}P/l. gives x counts per min. (The actual value of x is approximately 1800). For each experiment the reference solution for radioassay was a sample taken from the stock solution applied to the plants. In this way radioactive decay of ^{32}P and also small variations in counter efficiency were allowed for. Thus it was possible at any stage in the counting to relate the ^{32}P count of the stock solution to its schradan content.

If the solution to be assayed was an acid digest of a plant section then the ^{32}P count could be expressed only in terms of 'schradan equivalent', which is defined as the amount of schradan in the solution assuming all the ^{32}P is in this form. Thus all data expressed in this way are based on a ^{32}P count only and do not take into account any decomposition of the schradan which may have occurred prior to harvest.

If the solution is a filtrate after reflux treatment, counts were determined on the two phases after partitioning (see §6 (ii)). The ratio of the aqueous counts to the total were then a measure of the decomposition or breakdown of the schradan in the original filtrate. If the schradan content of the whole section was required, the 'schradan equivalent' of the filtrate and residue were determined and the true schradan content calculated from this using the breakdown data obtained. This procedure assumes that the breakdown in the filtrate and the residue is identical, and where extraction efficiencies approach the calculated, this assumption is justified. The different methods of expressing both 'schradan equivalent' and schradan are given in the relevant texts.

The general procedures adopted in a typical experiment may be summarized into three groups as follows:

Procedure *X*—Direct count on solution (e.g. leachate) giving schradan equivalent
Y—Acid digestion of section followed by counting giving schradan equivalent
Z—



Schradan content = (100 − % breakdown) × (Total 'schradan equivalent' in section).

In general leachates were subjected to procedure *X*, though in some cases partitions of these solutions were carried out.

Sections of the plant where activity was known to be low, or when information on the schradan content was not required, were subjected to treatment *Y*. Wherever desirable and practicable, however, procedure *Z* was applied.

The sum of the schradan equivalents in the sections at harvest can be expressed as a proportion of the schradan deposited at treatment. Where initial deposition data are lacking, the schradan equivalent in each section can be expressed as a proportion of the total recovered at harvest. By subtracting the schradan equivalent in the leachate from the total recovered at harvest, amounts in each section can be expressed as a proportion of that actually absorbed.

The authors are grateful to Miss E. Ward and Mr J. Sims for assistance in these investigations.

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APPENDIX

Experiments on the efficiency of gun spraying

Information was required on the amount of schradan solution remaining in the gun after spraying, and also the amount of sprayed solution which impacted a filter paper surface when a standard spraying procedure was followed. This involved keeping the gun nozzle about 1 in. from the surface to be sprayed, and simulating the movement required to give an even coverage to a leaf surface. Spraying was in each case continued until no more was emitted from the nozzle.

Before each treatment the gun was rinsed with the stock solution and sprayed to waste. A known volume of the radioactive stock solution was then placed in the gun cup, and sprayed on to filter paper as described above. The gun cup was then filled several times with aqueous non radioactive schradan and sprayed into a container to obtain a measure of the stock solution remaining in the gun after the original spraying. The filter paper was acid digested and radioassayed, and the gun washings made up to volume and also assayed. The data are shown in Table 1.

The results show an approximately constant amount, adhering to the walls of the gun cup. If, therefore, the gun cup is rinsed with the stock solution prior to a series of sprayings, the volume placed in the gun cup will actually be discharged from the nozzle each time. The sum of columns 2 and 3 shows that an average of about 4% of that discharged from the gun is not recovered due to failure of small particles to impact on the sprayed filter paper.

TABLE 1. *Evaluation of amount remaining in gun cup after spraying*

Vol. of solution placed in gun cup (ml.)	Recovery as percentage of vol. placed in gun: On sprayed filter paper	Recovery as percentage of vol. placed in gun: In gun rinsings
0·70	94·8	1·7
0·70	94·8	1·5
0·30	93·4	3·2
0·30	92·6	2·5

TABLE 2. *Evaluation of amount deposited on leaf and shield by spraying*

Plant species and no.		Volume of solution placed in gun (ml.)	% recovered on leaf + spray shield
Coleus	1	0·30	90·8
	2	0·30	90·7
	3	0·30	89·6
	4	0·75	88·1
	5	0·75	87·5
	6	0·75	85·2
	7	0·75	90·1
	8	0·75	89·6
	9	0·80	86·2
	10	1·82	90·4
	11	1·73	93·6
	12	1·23 (+wetter)	89·0
	13	1·45 (+wetter)	91·2
Broad beans	1	0·30	89·1
	2	0·30	93·3
	3	0·35	88·4
	4	1·19	87·3
	5	1·49	87·6
	6	0·86 (+wetter)	89·5
	7	1·09 (+wetter)	90·0
Apples	1	1·42	86·5
	2	1·35	89·0
	3	1·30 (+wetter)	86·0
	4	1·05 (+wetter)	88·4

To determine if the efficiency of impaction was a constant for the various leaf surfaces used in this work, a series of sprayings was undertaken in which the leaves were backed by 6 × 6 in. filter paper shields. Results given in Table 2 show that a constant proportion (89%) of the volume placed in the gun does impact the leaf or filter paper spray shield. The volume of solution deposited on the leaf surface is therefore taken to be 89% of the volume placed in the gun less the amount recovered from the spray shield.

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THE ABSORPTION, TRANSLOCATION AND BREAKDOWN OF SCHRADAN APPLIED TO LEAVES, USING ^{32}P-LABELLED MATERIAL

II. EVAPORATION AND ABSORPTION

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(With 1 Text-figure)

When ^{32}P-labelled schradan was sprayed on the leaves of apple stocks and seedlings, broad and runner beans, *Coleus* and chrysanthemums, some was absorbed, some evaporated and the rest remained for a considerable time on the leaf surface as a residue removable by aqueous leaching. Absorbed material is defined as that present on or in the plant and not removable by aqueous leaching at the time of harvest.

Evaporation losses even from glass slides were lower than theoretical expectation, from leaves they were much less.

It is possible that some breakdown of the schradan occurs within the cuticular layer. Comparisons of the absorption rates of upper and lower surfaces of leaves support the theory that absorption proceeds through the cuticle in preference to vapour phase entry through the stomata.

Temperature and illumination have important effects on absorption.

Young leaves have been shown to be generally more absorptive than the older leaves. Comparisons have also been made of the absorption by different species. Such data must be accepted with caution as, although physical conditions were the same for all the species within an experiment, the physiological stage of development of the different plant species will not be strictly comparable.

Introduction

This paper is concerned only with applications to leaf surfaces. If schradan is applied to a leaf surface it will be partly evaporated and partly absorbed. Unless both processes are governed by the same environmental factors, the proportion of evaporated to absorbed will vary with experimental conditions. The work described below was done to determine the importance of selected factors on the two processes.

We define absorbed material as that portion of the applied schradan in or on the plant which cannot be removed by aqueous leaching at the time of harvest. It is realized that such material may not have penetrated the cell walls but might be either adsorbed on, or absorbed in, the cuticular layer.

EXPERIMENTAL

A. *Evaporation*

The preliminary experiments on evaporation were made on glass slides (3 × 1 in.) subjected to air circulation and temperature changes. The slides received a deposit of 60 μg./cm.^{2} of ^{32}P-labelled schradan and, after drying, were exposed at 70 ± 3° F. in the air-flow apparatus previously described (Batt, Bennett & Thomas, 1954). The flow rate was 130 l./hr. ensuring a complete air change every 100 sec. Evaporation loss was assessed by thoroughly washing the slides with dilute acid and radio-assaying the solution. Details of the first two experiments and the results are assembled in Table 1, showing that evaporational losses can be appreciable particularly when the surface is close to the air jet.

TABLE 1. *Records of evaporation from glass slides*

Exp. no.	Slide no.	Distance from air inlet (cm.)	Direction of treated surface	% loss of initial deposit	
1	1	4	Facing inlet	96	After 48 hr.
	2	18	Facing inlet	85	
	3	28	Facing inlet	85	
2	1	2·5	Facing inlet	96	After 72 hr.
	2	5·0	Facing outlet	42	
	3	24·0	Facing inlet	53	
	4	26·5	Facing outlet	50	
	5	(Control slide on lab. bench)		14	

In a third experiment a deposit of 60 μg./cm.^{2} was placed in tinfoil cups (1·9 cm. diam. × 0·3 cm. high), which facilitated end-window counting and permitted a study of evaporation from the same surface over a period. Four groups of these cups, each with three replicates, were placed as follows: (*a*) in the light box at 60–70° F.; (*b*) in the counting room at 60–65°; (*c*) on the open bench in the glass-house at 30–55°; (*d*) as (*c*), but shielded from draught by an inverted beaker. The average daily evaporational losses over 15 days in these situations were: (*a*) 3 %, (*b*) 0·8 %, (*c*) 0·5 % and (*d*) nil. of the initial deposit.

To compare evaporational losses from slides with those from bean foliage, two glass slides (3 × 2 in.) and two bean leaves of the equivalent area were each sprayed on the upper surface with 0·3 ml. of a 0·3 % solution. In 48 hr. in the laboratory at 60–70° F., the glass slides lost 37 and 42 % of the original schradan deposit and the leaves 7 and 8·5 %, although 22 and 26·5 % was still present on the leaves in a leachable form. Even allowing for absorption of 71 and 65 %, evaporation from the bean leaves remained less than from the slides in this experiment.

In experiments with whole plants in the air-flow apparatus, 1 ml. of a 0·4 % solution was applied to the upper and lower leaf surfaces of two potted bean plants, the soil and the pots being wax-sealed. The plants were then placed for 72 hr. at 68–72° F., and given 18 hr. light daily, in the air-flow apparatus modified so that the air entered through a collar surrounding the stem at soil level. The air leaving the apparatus was passed through water in two sintered glass bubblers to recover the evaporated material. Results are given in Table 2.

Comparison with Table 1 shows that, under similar conditions, evaporation from bean plants was much less than from glass slides, even allowing for the absorption.

In another air-flow test, four sets of detached *Coleus* leaves were sprayed on both surfaces with a known volume of 0·4% solution and after drying were suspended vertically in the middle of the tower. Details and results are shown in Table 3.

Table 2. *Evaporational losses from beans under different air-flow rates*

Plant no.	Air-flow rate (l./hr.)	% of original deposit		
		Leachable	Absorbed	In gas bubblers (evaporation)
8B	132	7·7	90·9	0·2
9B	25	3·2	98·0	Nil

Table 3. *Evaporational losses from detached* Coleus *leaves*

<table>
<tr><th rowspan="2">Test no.</th><th rowspan="2">Temp. range (° F.)</th><th rowspan="2">Air-flow rate (l./hr.)</th><th rowspan="2">Duration of treatment (hr.)</th><th colspan="2">% of original deposit</th><th rowspan="2">% evaporation (by difference)</th></tr>
<tr><th>In leachate</th><th>In leaves</th></tr>
<tr><td>5K</td><td>68–80</td><td>45</td><td>45</td><td>37</td><td>58</td><td>5</td></tr>
<tr><td>12K</td><td>65–70</td><td>45</td><td>45</td><td>31</td><td>67</td><td>2</td></tr>
<tr><td>13K</td><td>65–75</td><td>132</td><td>40</td><td colspan="2">— 85 —</td><td>15</td></tr>
<tr><td>14K</td><td>65–75</td><td>28</td><td>40</td><td colspan="2">— 96 —</td><td>4</td></tr>
</table>

These results suggest that within the temperature range of these experiments air circulation may have been more important than temperature in promoting evaporation but, under similar air-flow rates, evaporation was greater at the higher temperature and occured more readily from *Coleus* than from beans.

In an experiment with apple stocks not exposed to artificial air-flow the plants were dipped (in September) in a 0·55% solution plus 0·25% wetter. When they were harvested after 60 days in the glasshouse (52–72° F.), about 20% of the schradan recovered was still present on the leaf surface in a water-leachable form. The presence of wetter may have had an effect on the retention of the schradan.

B. *Absorption*

1. *Position and status of absorbed schradan in leaves*

In experiments to determine the position of absorbed schradan in broad bean leaves, the leaves were water-leached, allowed to dry, then immersed in successive 75 ml. volumes of chloroform for known times, followed by a further aqueous leaching before radioassay. As the chloroform dissolved fractions of the cuticular layer, it was expected that radioassay of the chloroform solutions might reflect the progress of absorption of the insecticide in this layer.

Results of preliminary determinations on the leaves of three plants are shown in Fig. 1 in which the total schradan equivalent leached by the chloroform is expressed as a percentage of the total recovered, and plotted against time in seconds. The leaves from two bean plants (A and B) harvested in March, 2 days after treatment with 0·2 % stock solution, were subjected to seven 10-sec. immersions in successive volumes of chloroform. The third plant (C) harvested 3 days after treatment was subjected to three 30-sec. and four 1-min. immersions in successive volumes of chloroform. Fig. 1 shows that the amount leached by the chloroform increased during the first 90 sec., after which no further material was leached for the next 3 min. Further material was leachable, however, if the chloroform treatment was prolonged beyond this stage.

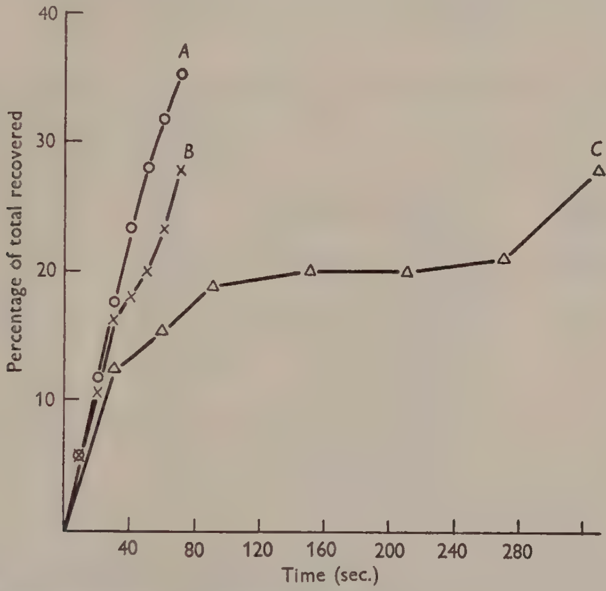

Fig. 1. The percentage of schradan equivalent leached by chloroform from broad bean leaves.

The rapid increase of schradan equivalent in the chloroform solution during the first 90 sec. may be a result of the dissolution of the cuticle and the liberation of the schradan within it. The smaller proportion leachable shown in curve *C*, compared with *A* and *B* during this time, may be attributed to the longer period between treatment and harvest resulting in more of the absorbed schradan penetrating below the cuticle. The failure to extract during the following 3 min. (curve *C*) is probably associated with the time interval necessary for extraction to occur following penetration of the tissue. Such an extraction process appears to begin about 4 min. after the initial immersion.

In a second experiment five bean plants with four or five leaves had all leaves sprayed with a 0·5 % solution in March, kept in the special laboratory, and harvested after 3, 7 and 14 days respectively. The treated leaves of plants harvested after 3 and 7 days were first water-leached and then, when dry, leached for 3 min. in chloroform, a period corresponding to the mid-point of the plateau for curve (*C*), Fig. 1. After the excess chloroform had evaporated, the leaves were again water-leached and then extracted by refluxing. The untreated part of the plant was acid digested. The treated leaves of the plant harvested after 14 days were subjected to the same treatment and the untreated part of the plant was extracted by refluxing. Breakdown of the schradan was determined in all solutions (see Part I). Chloroform leachates were evaporated at 50° C., taken up in water and subjected to alkali treatment before partitioning. The results are shown in Table 4, as a mean of two replicates in which the content of each fraction is expressed as a percentage of the total recovered at harvest.

TABLE 4. *Position and status of schradan in treated bean plants*

		Duration of treatment		
		3 days	7 days	14 days
1st aqueous leachate	% of total recovered	10·6	7·1	6·9
	Breakdown	31·1	39·5	43·1
Chloroform leachate	% of total recovered	35·5	24·8	15·5
	Breakdown	10·1	32·6	38·5
2nd aqueous leachate	% of total recovered	4·4	4·2	4·5
	Breakdown	11·4	29·9	31·3
Treated leaves	% of total recovered	49·4	63·9	56·9
	% extraction efficiency	97·1	97·8	95·6
	Breakdown	13·5	37·9	37·8
Rest of plant	% of total recovered	—	—	16·2
	% extraction efficiency	—	—	76·6
	Breakdown	—	—	73·8

The high values for breakdown observed in the 1st aqueous leachates are surprising. Analysis of the stock solution had indicated that only 94 % of the ^{32}P was present as schradan. Possibly the impurities were absorbed more slowly than the schradan. If this were so, the proportion of impurities on the leaf surface at harvest, would be high and the analysis of the 1st aqueous leachate would consequently show a breakdown figure even if none had occurred.

To check this an experiment was made with a much purer sample of radioactive schradan. Six bean plants and three *Coleus* were sprayed with a 0·2 % solution. Three of the bean plants were placed under 18 hr. flourescent light each day and harvested after 3 days. The other beans and the *Coleus* were placed in the special laboratory and harvested after 4 days. The procedure at harvest was the same as for the previous experiment.

Each value in Table 5 is the mean of three replicates and shows that in this series the breakdown in the 1st aqueous leachate was very low, so that the explanation suggested for the apparently high breakdown rates for the 1st aqueous leachates in Table 4 is probably correct.

Tables 4 and 5 demonstrate that chloroform leaching extracted large amounts of schradan or schradan equivalent not removed by water leaching. The 2nd aqueous leaching removed very little further material. With the exception of broad beans in daylight, the breakdown rates for the $CHCl_3$ leachate, the 2nd aqueous leachate (where present) and the treated leaves were of the same order within a plant.

TABLE 5. *Position and status of schradan in treated bean (B) and* Coleus *(K) plants*

		Light treatment and duration		
		Fluorescent 3 days	Daylight 4 days	
		B	B	K
1st aqueous leachate	% of total deposit	8·5	5·7	24·1
	Breakdown	<1	2·0	3·5
Chloroform leachate	% of total deposit	32·0	25·6	34·6
	Breakdown	10·6	4·0	<1
2nd aqueous leachate	% of total deposit	0·1	0·4	<0·1
Treated leaves	% of total deposit	42·4	44·6	7·6
	% extraction efficiency	100	99·6	98·1
	Breakdown	14·0	15·4	1·0
Rest of plant above soil level	% of total deposit	2·6	3·4	3·1
	% extraction efficiency	94	95	86
	Breakdown	38·3	33·7	3·6

These results do not give a clear picture of the position and status of schradan during absorption. If it is assumed that chloroform is extracting only from the surface layers, then the breakdown in the chloroform leachates suggests that breakdown of schradan occurs within the cuticle itself, or at its interface with the cell walls where there may be an enzyme system capable of breaking down the schradan. If, however, extraction from within the tissue has occurred, this extraction would be either of schradan itself or derived compounds which are chloroform-soluble at this stage. The fact that the breakdown in the leaf residue is slightly higher than that of the chloroform leachate would support a theory of the removal of chloroform-soluble substances from within the tissue, but the difference in the breakdown is not as great as might be expected in view of the relative amounts involved. In *Coleus* (Table 5), breakdown is extremely low throughout, the interesting point being the large amount removed by the 1st aqueous and chloroform leachates compared with that remaining in the treated leaves. The higher proportion which is water-leachable is discussed later in this section. While the percentage breakdown of this aqueous leachate is low, the large proportion involved is such that the possibility of schradan breakdown products occurring in a water-leachable form from leaf cuticle under certain conditions cannot be excluded. The high proportion present in the chloroform leachate compared with the small amount remaining in the treated leaves could be due either to decreased absorption by, or greater extraction from, the leaf tissue.

In all other experiments referred to in this paper, aqueous leaching only was used and any material not thus removed was regarded as absorbed.

2. *Absorption by upper leaf surface and by lower leaf surface*

The first experiment comparing absorption by upper and lower surfaces of mid-section leaves was made on *Coleus*, broad beans and apples in September. The upper surfaces of four leaf pairs on *Coleus* (12 in. high), four leaves on broad beans (18 in.) and twelve on apple stocks (2–3 ft.), were evenly treated with a 0·2% solution. A corresponding number of plants of each species received a comparable spraying on the leaf under surfaces. The treated plants were kept 3–4 days in the glasshouse (53–75° F.), and at harvest the sprayed leaves were leached and bulked with the rest of the shoot for acid digestion (for beans, the root was also included).

TABLE 6. *Comparison of upper and lower leaf surface absorption for* Coleus, *broad beans and apples*

Plant species	Surface sprayed	Time from treatment to harvest (hr.)	% of initial deposit		
			In leachate	Absorbed	Evap. (by diff.)
Coleus	Upper	75	31·4	48·9	19·7
	Lower	75	38·3	53·1	8·6
Broad beans	Upper	72	9·7	87·6	2·7
	Lower	72	10·9	83·3	5·8
Apples	Upper	94	87·8	6·0	6·2
	Lower	94	61·4	32·5	6·1

Table 6 in which each value is the mean of three replicates shows that in *Coleus* and beans the absorption by both surfaces is similar, but in apples the lower surface is far more absorptive than the upper.

In a second experiment made in July on the mid-stem leaves of nine chrysanthemum plants, eight leaves of approximately equal total area on each plant were sprayed with a 0·27% solution. 1 ml. per plant was applied to the upper leaf surfaces of plants 1–3, the same amount to the lower leaf surfaces of plants 4–6, and 2 ml. per plant to both leaf surfaces of plants 8–9. The plants were then kept 7 days in the glasshouse (63–85° F.). At harvest the treated leaves were leached and acid digested (Table 7).

These results, means of three replicates, show that the lower surface of chrysanthemum leaves is, as in apple, much more absorptive than the upper surface. Although translocation loss was not separately determined, other comparable experiments have shown that this amount is unlikely to exceed 5% of the initial deposit.

A third short-term experiment was carried out on chrysanthemums and broad beans in January. A 0·3 % solution was used to spray four leaves detached from chrysanthemum plants and three leaves on bean plants. Half of each batch was treated on the upper leaf surface and the rest on the lower. After spraying, the leaves were allowed to dry in the dark and kept at 70° until harvest $2\frac{1}{2}$ hr. later.

TABLE 7. *Upper and lower leaf surface absorption for chrysanthemums after 7 days*

	% of initial deposit		
Surface sprayed	In leachate	Absorbed	Evaporation and translocation (by diff.)
Upper (1 ml.)	19·6	39·0	41·4
Lower (1 ml.)	9·2	78·3	12·5
Both (2 ml.)	15·0	56·4	28·6

TABLE 8. *Upper and lower leaf surface absorption for broad beans and chrysanthemums after $2\frac{1}{2}$ hr.*

		% of initial deposit		
Plant species	Surface sprayed	In leachate	Absorbed	Evap. (by diff.)
Beans	Upper	85·3	15·8	−1·1
	Lower	83·0	18·4	−1·4
Chrysanthemums	Upper	98·6	4·9	−3·5
	Lower	83·0	21·5	−4·5

At harvest the treated leaves were leached and digested. The results (Table 8), mean of three replicates showed little difference between the amounts absorbed by the upper and lower leaf surfaces of beans. The scatter in the results is caused by the fact that initial absorption is rapid, and so small differences in absorptive rates tend to be exaggerated in a short term experiment. In chrysanthemums, the upper leaf surfaces were again far less absorptive than the lower. The negative values given in the final column indicate that more was recovered than corresponds to the calculated impaction figure.

These results from detached leaves, in which the stomata are probably closed, suggest that entry is through the cuticle. The similar absorption rates for both surfaces in *Coleus* (Table 6) also indicate that vapour phase entry through the stomata cannot be important, since *Coleus* have no stomata on the upper surface.

Table 6 shows that the ratio between evaporational loss and the water-leachable fraction varied considerably between different species kept in the same environmental conditions.

3. *Relative effects of light and heat on absorption*

In an experiment in July a set of apple stocks was treated in the morning and another in the evening of the same day. Both sets were conditioned for 12 hr. and at the time of morning application the other plants were brought into daylight until treated in the evening. In all other respects the two sets were treated alike. The plants were dipped in a 0·53 % solution and harvested after 72 hr. in the glasshouse (65–85° F.). The results indicated that the plants treated in the morning had absorbed more.

The effect of light immediately preceding and following treatment was studied on runner beans, broad beans and *Coleus* which were sprayed with a 0·5 % solution. For 36 hr. before spraying half the plants of each species were given fluorescent light for 18 hr. each day and the other half kept in the dark. After applying the solution each of these two sets of plants was divided into two groups, one maintained under the same light conditions, and the other in darkness for the 72 hr. (60–70° F.). (Table 9 mean of two replicates as a percentage of total recovered at harvest.)

Table 9. *The effect of light before and after treatment on absorption by broad beans, runner beans and* Coleus *harvested 72 hr. after treatment*

Exposure treatment		Broad beans	Runner beans	*Coleus*
Light → Light	% absorbed	88·4	86·7	73·7
	% leachable	11·6	13·3	26·3
Dark → Light	% absorbed	90·1	91·8	77·7
	% leachable	9·9	8·2	22·3
Light → Dark	% absorbed	74·7	56·5	53·2
	% leachable	25·3	43·5	46·8
Dark → Dark	% absorbed	80·4	58·5	47·7
	% leachable	19·6	41·5	52·3

All species absorbed more when light followed application, irrespective of the pre-application treatment. Within each species there was an indication that plants exposed to light after the application absorbed more if previously kept in the dark. The reduction in the proportion absorbed when darkness followed spraying was smaller in broad beans than in runner beans or *Coleus*.

To examine the effects of varying the periods of light treatment after spraying, chrysanthemum plants were sprayed in April and then given one of the following sequences of treatment, each of which started with a light period and was carried out at 63–75° F.:

(*a*) Plants given 18 hr. light each 24 hr. ('standard illumination'), and harvested at 4, 8, 24, 48, 96, 192 and 384 hr. after spraying.

(*b*) Plants given initial exposures of 1, $2\frac{2}{3}$, 4 or 8 hr. light, and harvested 48 hr. after spraying.

(*c*) Plants given initial light exposures as in (*b*) followed by 47 hr. darkness, then treatment (*a*) and harvested 96 hr. after spraying.

The results are given in Table 10*a*, *b* and *c*, in which the fraction absorbed is expressed as a percentage of the total recovered. Each value is the mean of three replicates.

TABLE 10*a*. *Absorption by chrysanthemums given* 18 *hr. light in each* 24 *hr.* ('*Standard illumination*')

Interval from spraying to harvest (hr.)	4	8	24	48	96	192	384
% absorbed	21·6	36·9	63·1	85·3	91·7	92·5	92·4

TABLE 10*b*. *Absorption by chrysanthemums harvested* 48 *hr. after spraying*

Treatment. Hours in light and dark	1L & 47D	2⅔L & 45⅓D	4L & 44D	8L & 40D	'Standard illumination' control
% absorbed	45·7	47·9	45·6	56·7	85·3

TABLE 10*c*. *Absorption by chrysanthemums harvested* 96 *hr. after spraying*

Treatment. Hours in light and dark	1L & 47D and *N to 96	2⅔L & 47D and *N to 96	4L & 47D and *N to 96	8L & 47D and *N to 96	'Standard illumination' (*N) control
% absorbed	81·6	87·9	84·2	85·6	91·7

* N refers to standard conditions of illumination (as in Table 10*a*).

Table 10*a* shows that absorption reached its maximum in 2–4 days, after which little, if any, further absorption occurred. Table 10*b* shows that for plants harvested 48 hr. after spraying there was little difference in the amount absorbed when the initial exposure to light was increased from 1 to 4 hr. An initial exposure of 8 hr. however, increased absorption, but not to the level reached under standard illumination. Table 10*c* showed that plants given the treatment of those in Table 10*b* followed by standard illumination for the remainder of the 96 hr. showed an increased absorption, and the variations resulting from the initial light treatments were greatly reduced.

In an experiment on beans carried out in late summer, harvests were carried out at intervals between 6 hr. and 4 days. The plants after being sprayed at 10 a.m. with 0·27% solution were placed in the glasshouse (55–75° F.).

Table 11 shows that 85% of the initial deposit was absorbed by the end of 48 hr. The fact that no absorption occurred between 10 p.m. and 10 a.m. (i.e. 12 and 24 hr. after spraying) might appear to be an effect of darkness, but the results given in Table 10*b* for chrysanthemums show that darkness reduced but did not inhibit absorption.

In another experiment it was found that broad beans in darkness absorbed during 72 hr. about 85% of the spray deposited, compared with 90% under fluorescent light. For runner beans the figures were 75% in darkness and 86%

under fluorescent light. Both sets of plants were kept for a period of 40 hr. in darkness at 65–75° before spraying.

As light was not apparently an essential factor in absorption, an experiment was designed to observe the relative effects of temperature and light. Two dark and two light glass-walled compartments were constructed and temperature in one light and one dark compartment was controlled at 80° F., and in the other pair at 50–60° F. (see Part I, §4).

Table 11. *Absorption and evaporation data in beans*

Interval between treatment and harvest (hr.)	% of original deposit	
	Leachate	Absorbed
6	63·8	39·1
12	34·7	61·4
24	34·4	58·8
48	9·5	84·2
96	3·2	84·2

Table 12. *Absorption by beans under different light and temperature conditions* (*mean of two replicates*)

Time from treatment to harvest (hr.)	Conditions							
	Light—Warm		Dark—Warm		Light—Cold		Dark—Cold	
	% of intial deposit							
	Leachable	Absorbed	Leachable	Absorbed	Leachable	Absorbed	Leachable	Absorbed
7	27·8	68·6	30·9	64·4	91·4	11·0	94·1	10·0
24	13·3	77·7	17·6	73·5	47·9	54·2	65·3	36·8
36	6·4	89·5	12·7	80·7	43·2	56·2	50·6	48·2
48	5·7	86·4	13·3	74·6	25·8	72·8	25·8	75·7
72	4·5	88·7	12·5	79·8	11·7	89·8	28·2	75·2

Bean plants with three open leaves were sprayed with 0·4 ml. of a 0·3 % solution in February and immediately placed in each of the compartments. Harvesting was carried out at intervals between 7 and 72 hr., the treated leaves leached and the whole plant, including the roots, acid digested. There was an average of 3 hr. sunshine per day.

Table 12 shows that temperature was more important than light in influencing the speed of absorption during the early stages. After 48–72 hr., however, the differences are lessened by the cessation of absorption at the higher temperature after 36 hr. Thus absorption in beans at 80° F. seems to follow an initially steep exponential curve whilst at the lower temperature it increases linearly. The fact that the ultimate absorption figures are higher in the light than in the dark would suggest that light increases the plant's absorptive capacity. It is possible, however, that this higher absorptive capacity may not be due to the positive action of light but rather to the effect of prolonged darkness adversely affecting the other plants.

These factors of temperature and light may not necessarily affect all plants in the same way or to the same degree. For broad beans temperature appears to be the dominating factor, particularly during the early stages (Table 12), whilst for chrysanthemums (Table 10), and *Coleus*, runner beans and possibly broad beans (Table 9), light increases absorption at the temperatures prevailing in these experiments.

4. *Age of leaf as a factor affecting absorption*

To determine the relative absorption of schradan by leaves of different age, experiments were carried out with chrysanthemums in June and with apple stocks in September. Each plant was envisaged as horizontally divided into three sections, approximately equal in leaf numbers, and one section only was sprayed. In the chrysanthemum plants, the lower, middle and upper sections had eight, eight and six leaves respectively. The required sections of three plants were sprayed with a 0·2% solution, then were placed in the glasshouse (51–81° F.) and harvested after 7 days. At harvest the treated leaves were leached and the whole plant radioassayed.

TABLE 13. *Absorption by chrysanthemum leaves on different sections of the plants 7 days after treatment (mean of three replicates)*

Section of plant treated	% of initial deposit In leachate	Absorbed	Evap. (by diff.)
Lower	15·1	72·3	12·6
Middle	27·6	60·2	12·2
Top	15·0	82·8	2·2

TABLE 14. *Absorption by apple leaves on different sections of the plant after 14 days*

Section of plant treated	% of initial deposit In leachate	Absorbed	Evap. (by diff.)
Lower	42·2	37·7	20·1
Middle	43·9	35·4	20·7
Top	31·8	48·9	19·2

Table 13 shows that in 7 days the youngest leaves absorbed most and the mid-section leaves least. Since similar proportions were leachable from the youngest and oldest leaves, while less was evaporated from the youngest leaves, it would appear that absorption was not only greater in the youngest leaves but also quicker. For lower and mid leaf treatments, evaporation was similar but more leachable material was present on the middle leaves.

In the apple experiment, there were four replicates, and after spraying with a 0·2% solution, the plants were placed in the glasshouse (50–78°) for 14 days.

Table 14 suggests that, as in chrysanthemums, the youngest leaves were the most absorptive over this period, but the difference in the proportion absorbed by the lower and middle leaves was less marked than in chrysanthemums. Evaporational losses from all leaves was of the same order.

5. *Effect of concentration of solution on absorption*

The effect of the concentration of applied solution on absorption was studied on apple stocks treated in July by dipping their mid-section leaves in 0·53 and 0·053% solution (plus wetter). After treatment the stocks were placed in the glasshouse and harvested after 8 hr., 3 and 7 days. There were three replicates for each treatment.

TABLE 15. *Effect of solution concentration on absorption by mid-section apple leaves*

Interval between treatment and harvest	Absorbed as % of total recovered at harvest	
	For 0·53% solution	For 0·053% solution
8 hr.	26·2	14·7
3 days	50·6	35·3
7 days	62·6	36·0

Table 15 shows that at each harvest a greater proportion was absorbed from the solution of the higher concentration. Other experiments on beans and apples have shown the same trend. However, the surface density of schradan on the leaf is probably more important than the concentration of the applied solution, and the experiment is interpreted as showing that application of 10 times more schradan to the leaf surface results in considerably more than 10 times the amount of schradan being absorbed.

6. *Absorption by different plant species*

Although the results already given indicate that the amounts absorbed by different plant species vary considerably, comparisons are of little value unless made between plant species maintained in the same controlled environment. Even these comparisons may be affected by differences in the stages of growth of the plants compared. In this section some of the comparative data obtained on plant species are reviewed.

Table 6 shows that broad beans are more absorptive than *Coleus* which in turn absorb more than apples. As these results also give the proportions leachable and evaporated, it is evident that the relative amounts absorbed are not determined by the amounts available, but represent a true difference between the absorptive powers of the three species.

Another comparison can be made between broad beans, runner beans and *Coleus*.

Table 9 shows that where plants were placed in the light after treatment, irrespective of the pretreatment conditions, runner beans and broad beans absorbed about the same amount, with *Coleus* rather less. When the plants were placed in the dark after treatment, irrespective of pretreatment, absorption in all cases was reduced. This reduction was less marked in broad beans whilst in *Coleus* and runner beans absorption was more nearly equal. These results which are based on a percentage of the total recovered at harvest do not take into account evaporational losses, which have been found to be greater for *Coleus* than for the two species of beans, so the true difference between the absorption by *Coleus* and that by beans is even greater than that shown.

In other experiments, the relative absorption of these three crops has been compared over periods of up to 7 days. Throughout, the absorption by broad and runner beans has exceeded that of *Coleus*. The proportion present in a leachable form on the leaf surfaces has been invariably greater for *Coleus* than beans.

Discussion

Using the value of 2×10^{-4} mm. mercury for the vapour pressure of schradan at 24° C. (determined by an effusion technique), and making certain assumptions, Heath & Llewellyn (1953) have calculated a value of $1{\cdot}5 \times 10^{-3}$ mg./hr./cm.^{2} for the theoretical evaporational loss from a plane surface at 15° C., provided the surface is completely covered with a layer of schradan at least two or three molecules thick, and also that the movement of air is sufficient to maintain the concentration of schradan in the vapour phase below its saturation value ($8{\cdot}4 \times 10^{-4}$ mg./l.).

In our experiments the initial deposits on the sprayed surfaces have varied widely according to the concentrations of solutions applied (1–0·005 %). Assuming a value of about 50 sq.Å. for the cross-section of a schradan molecule, the initial deposit of schradan after spraying with, say, a 0·2 % solution under our experimental conditions would correspond to a uniform film of about 8–12 μg./cm.^{2} or 80–120 molecular thicknesses. If therefore more than about 3–5 % of this is found to be still present in a water-leachable form on the leaf surface at harvest, the evaporation should have been according to physical laws provided the temperature was 15° C. or higher.

Evaporational losses, however, are found to be lower than would be anticipated, even on glass slides suspended in a glass tower in which there is a complete air change every 100 sec. Where comparisons are possible (see p. 485) evaporational losses from leaves were shown to be lower than from glass surfaces.

An initial deposit of about 19 μg./cm.^{2} was placed on bean leaves and glass slides, and during the first 48 hr. glass slides lost 7·6 μg./cm.^{2} and the leaves 1·4 μg./cm^2. Absorption had accounted for 13 μg./cm^2. but there was still 4·6 μg./cm.^{2} leachable from the leaf surface. Thus, assuming a fairly uniform coverage it would appear that although the rate of evaporation from the glass slides was about 5 times that from bean leaves, at the end of the experiment the leaves still retained a surface film of nearly 50-molecular thickness. The low rate of loss from glass slides in this experiment may well be due to insufficient air turbulence since it has been shown that rapid air change induces much greater losses.

This explanation does not account for the 5-fold lowering of the evaporation rate on the bean leaves. This phenomenon which is common to all leaf surfaces, may well be associated with lipoid solubility of the schradan.

Frey Wyssling (1948) describes the cuticular layer as composed of a mixture of the four cell-wall constituents cellulose and pectins, which are hydrophilic compounds, cutin which is semi-hydrophobic and cutin waxes which are lipophilic. On the inner face of this cuticular layer the cellulose and pectins predominate while to the outside the waxes are predominant. The outer layers may be composed of cutin and wax only. The conception of a homogenous film of schradan remaining on the leaf surface may therefore be misleading since it is possible that not only does the schradan dissolve or permeate some of the lipophilic substances of the cuticle but some of the lipophilic substances may dissolve in the schradan. If a process of this nature does occur it would serve to retard the evaporation of schradan from the leaf surface. The effectiveness of this retardation may depend on the extent to which this mutual penetration has occurred and the location of the various constituents; whatever mechanism is involved it does not prevent the removal of the material by a very simple aqueous leaching.

Evaporational losses are always lower than would be anticipated. The generally higher losses from *Coleus* and chrysanthemum leaves may be associated with the increase of surface area due to the leaf hairs as well as to differences in the cuticles.

While information on the breakdown of the material which is water-leachable from the leaf is incomplete, the possibility of breakdown occurring under the influence of some cuticular complex cannot be excluded, though the extent of breakdown is likely to be small. The much larger fraction which is removed from the leaf by chloroform treatment has a breakdown only slightly lower than that of the fraction which remains within the tissues after this treatment. Whilst in our experiments the possibility of extraction from within the cell membranes cannot be eliminated, it would appear from the amounts involved and the breakdown data observed, that even if this did occur some breakdown of schradan must take place in the cuticular layer. Hall, Stohlman & Schechter (1951) have claimed that the active cholinesterase inhibitor produced from schradan in a species of bean is extractable by chloroform from the aqueous extract. As the chloroform solutions in our experiment were alkali-treated before partitioning, any such cholinesterase inhibitors present would have been broken down into substances partitioning in favour of alkali from chloroform. Thus the principal source of the breakdown shown to occur in the chloroform could be this active compound.

On the other hand, the structure of the cuticle would indicate the possibility of the dissolution of the lipophilic substances by chloroform thus liberating islands of hydrophilic compounds with which the alkali-soluble breakdown products are in close association.

Breakdown in the cuticular layer may be expected to occur most readily in the inner region where the proportion of cellulose and pectins is greatest. It may in fact occur only at the interface with the cell walls.

The main modes of entry of material into plant foliage are cuticular (Weaver & De Rose, 1946; Fogg, 1948), by liquid penetration of the stomata by some of the petroleum oils, the rate of penetration depending on surface tension and viscosity (Knight & Cleveland, 1934); and by vapour phase entry through the stomata. Heath & Llewellyn (1953) have adduced evidence supporting the cuticular mode of entry for some organo-phosphorus compounds including schradan. The work discussed in this paper adds support to this theory. The absorption by the upper and lower leaf surfaces of *Coleus* was found to be of the same order, while the same type of behaviour was observed with broad beans. The observations on *Coleus* is of particular interest as the upper surface of the leaves have no stomata. In chrysanthemums and apples the lower surface is more absorptive but this may well be explained by differences in the nature of the cuticular layer of the two surfaces. Quite rapid absorption has also been observed in leaves after removal from the plant when stomata would be closed.

Thus whilst some plants show similar absorption for their upper and lower leaf surfaces, in others the lower leaf surface is the more absorptive. In none of the plants listed has the upper surface been found the more absorptive. From the practical standpoint therefore it might well prove beneficial to pay attention to lower leaf surface application from which absorption will be as good as, if not better than, from the upper leaf surface, and from which losses by rain leaching, etc., would not be as great.

It would appear from the results of our experiments that light and heat can be important factors in absorption. The effect of energy in the various spectral regions of the incident radiation is not easily separated. Davson & Danielli (1952), suggest that the plasma membrane is a potential barrier, to penetrate which the molecules must possess a critical energy of translation, so that when the critical energy required is large, a rise of temperature may cause a larger number of molecules to permeate the membrane. Heath & Llewellyn (1953) concluded that the process of absorption was sensitive to infra-red and visible radiation but that this was not necessarily an effect on the whole plant but due to a temperature gradient set up near the leaf surface which increased absorption. It has been found in the present work (Table 12) that absorption in beans can be greatly increased by raising the temperature, even when the plants are screened from any direct radiation, this being an effect on the rate and not the capacity of absorption. Thus whilst at the higher temperatures daylight does not increase the rate of absorption, at the lower temperature it does. At both temperatures light seems to increase absorptive capacity. It may well be that optimum conditions exist for all plant functions, two of the major factors for which would be light and temperature. At optimum conditions the cell membranes are in their most permeable state. Such optimum conditions will vary from species to species (Comm. Plant Irradiation Ned. St Verl 1953) and when the conditions for a species are sub-optimal, the relative effectiveness of any factor is assessed by its ability to make the conditions approach optimum. It is for this reason that in some of the experiments there was more absorption in light than in darkness (Tables 10*b* and 10*c*) while in others absorption was increased by a rise of temperature (Table 12).

The cause of the variation in absorption with age of the leaf is not known.

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THE ABSORPTION, TRANSLOCATION AND BREAKDOWN OF SCHRADAN APPLIED TO LEAVES, USING ^{32}P-LABELLED MATERIAL

III. TRANSLOCATION AND BREAKDOWN

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(With 1 Text-figure)

A study of translocation of ^{32}P-labelled schradan in apples, chrysanthemums, broad and runner beans and *Coleus*, has shown light to be an important factor.

The main direction of translocation is upwards but some schradan does travel downwards. In apples translocation takes place mainly in the phloem but some may take place in xylem particularly upwards.

No major difference has been observed in the ratio translocated/absorbed for *Coleus*, beans and chrysanthemums but in apples this proportion is greater.

The breakdown of schradan into non-chloroform extractables varies between the plant species and is far greater in beans than in *Coleus*.

In chrysanthemums and apples the breakdown appears similar throughout the plant whilst in beans and possibly *Coleus* the breakdown in the untreated sections is higher.

Concentrations necessary for the kill of certain species of aphides are given.

INTRODUCTION

This paper deals with the translocation and breakdown of schradan applied to leaves. Systemic insecticidal efficiency is determined by the rate, amount and direction of such movement. It is known that a plant treated with schradan loses its insecticidal properties after a period of time, (Bennett & Thomas, 1953). One of the factors determining the duration of toxicity is the breakdown of the schradan into non-insecticidal products. In our investigations only the extent of degradation and not the identity of these products has been considered.

These experiments provide information on factors affecting translocation and breakdown and their variation between species.

EXPERIMENTAL

In the trials described below, different sections of the plants used are indicated by letters, as shown in Fig. 1.

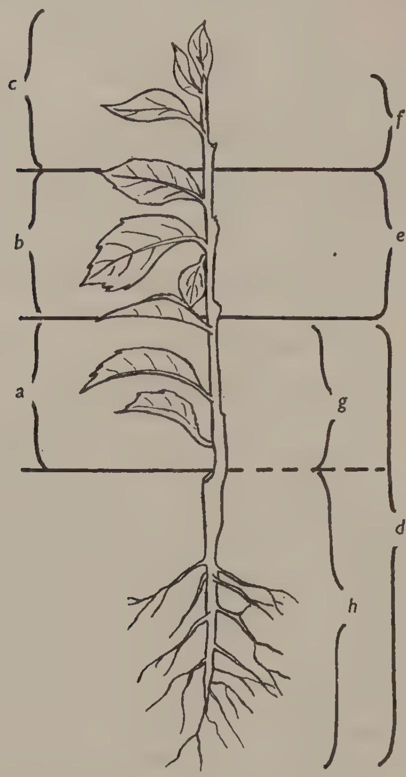

Fig. 1. The scheme: *a*, lower leaves; *b*, middle leaves; *c*, upper leaves; *d*, root, plus stem portion bearing *a*; *e*, stem portion bearing *b*; *f*, stem portion bearing *c*; *g*, stem portion bearing *a*; *h*, 2-year-old wood and root.

EXPERIMENTS ON APPLE

Translocation of schradan from mid-stem apple leaves

In July, nine apple stocks, each 3 ft. high and with approximately thirty-six leaves, were treated with 0·53 % solution. Nine comparable stocks were treated at the same time with 0·053 % solution. The treatment was given by dipping the twelve middle (*b*) leaves of each stock. The plants were then kept in the glasshouse (70–89° F.) and harvested in equal numbers after 8, 72 and 168 hr. Each plant was analysed in six of the sections shown in Fig. 1. At each harvest the *b* leaves were leached and the amount absorbed expressed as a percentage of the total recovered at harvest.

Table 1 shows that only slight translocation from the middle leaves occurred after 8 hr., about 6–10 % after 3 days, and 14–16 % after 7 days. The upper leaves (*c*) received more from *b* than did the lower leaves (*a*), but in the stem-and-root section at the two later harvests, the major accumulation was in the lower sections, the order being $d>e>f$. Separate harvest of g and h (Fig. 1) showed that after 10 days 85–90% of the amount in the d section was in the 2-year-old wood and roots.

TABLE 1. *Translocation of schradan equivalent in apple stocks following middle leaf* (*b*) *application* (*using two concentrations of stock solution*). *Each value is mean of three replicates*

Carrier level	Interval between treatment and harvest (hr.)	Amount absorbed as % total recovered	% of amount absorbed in sections					
			a	*b*	*c*	*d*	*e*	*f*
0·53	8	26·2	0·01	99·6	0·1	0·0	0·2	0·0
0·053	8	15·3	0·3	98·6	1·0	0·0	0·07	0·07
0·53	72	50·6	0·4	90·7	2·6	3·3	2·7	0·2
0·053	72	35·6	0·5	94·3	2·0	1·4	1·7	0·1
0·53	168	55·9	0·9	84·4	5·5	5·6	3·1	0·5
0·053	168	36·4	1·1	86·6	5·3	4·3	2·3	0·4

A comparison between the two carrier levels at the 3-day and 7-day harvests showed that the proportions in the various sections were similar for both levels, although the absolute amount was about 10 times greater for the higher level. It therefore appears that the proportion translocated to any one section is irrespective of the total available for translocation.

Translocation from lower, middle and upper apple leaves

In June, twenty-seven apple seedlings, each 18–24 in. high with approximately twenty-four expanded leaves were divided into three groups of nine and treated with 0·63% solution. In the three groups, the lower (a), middle (b) and upper (c) leaves respectively were treated at 10 a.m. by dipping. The plants were then kept in the glasshouse (58–82° F.) and harvested in equal lots after 8, 72 and 144 hr. The treated leaves were leached and analyses done on the three leaf sections (a, b and c in Fig. 1) and on a combined stem-and-root section, corresponding to $d+e+f$.

Table 2 agrees with Table 1 in showing that little translocation occurred in the first 8 hr., and that subsequently the upward translocation from b leaves to c was much greater than the downward movement from b to a. Translocation from a to c approximately equalled that from a to b but the translocation from c to the remaining leaves remained very low throughout. The totals absorbed in the stem and roots were approximately equal to the corresponding combined amounts for d, e and f in Table 1.

Translocation from upper and middle leaves: long-term experiment on apple stocks

In an experiment maintained for 60 days, apple stocks were treated on 1 September. The stocks had 40 to 50 leaves of which 15 to 16 in either the b or the c region were dipped in a 0·55 % solution. The plants were kept in a glasshouse for 39 days (55–75° F.) or 60 days (52–72° F.), with an average of 3 hr. sunshine per day. At harvest each plant was analysed in the six sections shown in Fig. 1. After 39 days for plants with the upper leaves treated, only the treated leaves were partitioned, while for *b* treated plants *b* and *c* leaves were partitioned. After 60 days only the *b* treated leaves showed sufficient radioactivity to permit partitioning.

The results, expressed both on a proportional and a concentration basis, Table 3, show that translocation from the *b* leaves after 39 days in Sept.–Oct. was of the same order as after 7 days in July or 6 days in June (see Tables 1 and 2), and that the distribution through the plant was similar. After 60 days there was a pronounced increase in the amount accumulated in the *d* and *e* sections, giving concentrations equivalent to that in the *c* leaves; this may be associated with autumn storage processes.

Translocation from the *c* leaves remained low but was rather more after 39 days in Sept.–Oct. (Table 3) than after 6 days in June (Table 2).

Table 4 shows that the extraction efficiencies were low compared with those for shorter-term experiments, probably because of fixation of the schradan decomposition products in forms not extractable by water. No further breakdown occurred between 39 and 60 days in the *b* treated leaves, this may be associated with the time of the year. The concentration of schradan after 39 days, expressed as μg./g. fresh weight, was more than twice as much in the treated *c* leaves than the treated *b* leaves. This was partly due to the lower breakdown in *c* leaves but the more important factor was the small amount translocated from these upper leaves.

Translocation from middle leaves of apple stocks: effect of concentration of solution

Two apple stocks 24 in. high had the eight middle leaves sprayed in May with 1 and 0·25 % solutions respectively. The plants were placed in the glasshouse (50–75° F.: mean daily sunshine, 5 hr.) and harvested after 7 days. Analyses were made on the sections shown in Fig. 1, *d* being subdivided into the stem portion *g* bearing the *a* leaves and the remainder *h*.

The results (Table 5) show that, of the amount absorbed from the 0·25 % solution, 24·5 % was translocated compared with 7·5 % of the amount absorbed from the stronger solution. The proportional distribution is the same in *a*, *b*, *c* and *f* for both concentrations but is five times greater in *g* and *h* following the 1 % application, with a corresponding reduction in *e*.

The concentration of schradan equivalent is greater for the plant sprayed with 1 % solution in all sections except *e* but the more efficient translocation in the plant sprayed with 0·25 % solution much reduces this effect. The more efficient translocation in the plant treated with the lower concentration contrasts with the data given in Table 1, where the concentrations used were 0·53 and 0·053 %.

TABLE 2. *Translocation of schradan equivalent in apple seedlings following applications in June to lower, middle and upper leaves respectively. (Each value is mean of three replicates)*

Leaf section treated	Interval between treatment and harvest (hr.)	% of amount absorbed in leaf sections			% of amount absorbed in stem and roots
		a	*b*	*c*	
Lower (*a*)	8	99·3	0·3	0·2	0·2
Middle (*b*)	8	0·03	98·6	0·1	1·2
Upper (*c*)	8	0·06	0·1	99·3	0·5
Lower (*a*)	72	91·1	1·7	1·2	6·0
Middle (*b*)	72	0·3	90·7	2·6	6·4
Upper (*c*)	72	0·05	0·3	93·9	5·8
Lower (*a*)	144	83·0	3·1	3·8	10·1
Middle (*b*)	144	0·7	77·5	10·0	11·8
Upper (*c*)	144	0·0	0·1	97·0	2·9

TABLE 3. *Distribution and concentration of schradan equivalent in apple stocks treated in September: long-term experiment. (Each value is the mean of three replicates)*

Leaf section treated	Interval between treatment and harvest	Amount absorbed as % of total recovered	% of amount absorbed in						Concn. of schradan equivalent in µg./g. fresh wt.					
			a	*b*	*c*	*d*	*e*	*f*	*a*	*b*	*c*	*d*	*e*	*f*
c	39 days	85·0	0·4	0·4	91·0	2·4	2·1	3·7	5	2	421	4	10	37
b	39 days	64·0	1·1	80·5	5·8	8·9	3·2	0·5	7	251	12	7	8	2
b	60 days	76·1	0·8	70·0	5·1	18·5	5·1	0·5	7	264	14	17	16	3

TABLE 4. *Mean values for extraction efficiency, breakdown data and schradan concentration of b and c leaves for apple stocks (see Table 3). (Each value is the mean of three replicates)*

Leaf section treated	Interval between treatment and harvest	*b* leaves			*c* leaves		
		% extraction efficiency	% break-down	Conc. of schradan µg./g. fresh wt.	% extraction efficiency	% break-down	Conc. of schradan µg./g. fresh wt.
c	39 days	83·6	*	—	85·5	62	163
b	39 days	82·1	75	63	83·2	(69)	(4)
b	60 days	79·2	74	69	—	—	—

* Counts too low for partitioning.

( ) Values in brackets based on rather low counts.

TABLE 5. *Distribution of schradan equivalent in apple stocks and records of extraction, breakdown and concentration in leaves 7 days after application to b leaves in May*

		Sections						
					d			
		a	*b*	*c*	*g*	*h*	*e*	*f*
Section content as % of absorbed	1 %	0·2	92·5	4·8	0·3	0·3	1·3	0·6
	0·25 %	0·4	75·3	15·8	0·2	0·2	5·9	2·2
% distribution of translocated schradan equiv.	1 %	2·7	—	64·0	4·0	4·0	17·3	8·0
	0·25 %	1·6	—	64·0	0·8	0·8	23·9	8·9
Concn. of schradan equiv. as μg./g. fresh wt.	1 %	7	2789	277	15	2	57	77
	0·25 %	5	619	187	3	0·2	67	50
% extraction efficiency	1 %	86	94	96				
	0·25 %	M.v.	91	96				
% breakdown	1 %	30	26·5	21				
	0·25 %	(30)	33	26				
Schradan concn. in μg./g. fresh wt.	1 %	5	2050	218				
	0·25 %	(3)	414	138				

M.v. Missing value.
( ) Value in brackets based on rather low counts.

The proportion absorbed, as a percentage of the total recovered was 96·4 and 91·8 for plants treated with 1 and 0·25 % respectively.

Translocation to roots and new growth: comparison of effects of fluorescent light and daylight

The effect of light was studied by spraying in June all the expanded leaves on six apple stocks 12 in. high, using a 0·2 % solution. Three plants were kept in the glasshouse (55–89° F.) and three under fluorescent light for 18 hr. daily (73–80° F.). The spray treatment had some phytotoxic effect on the leaves, particularly those kept under fluorescent light. After 15 days the treated leaves were leached, bulked with the section of stem on which they grew, extracted and partitioned. The new growth at the stem apex was harvested separately, as were the roots, and both were acid-digested.

The results (Table 6) show greater absorption under the fluorescent light (probably because of the higher rate of evaporation from the plants in the glasshouse). The 2–3 times greater concentration of schradan in the treated strata of fluorescent light-treated plants is the result of greater absorption and less translocation.

The proportion translocated to the new growth was, however, about twice as much for the plants in daylight making the total concentration of schradan-equivalent in this region approximately the same in both sets of plants. Translocation to the roots in the daylight-treated plants was 5 times that in the others, so the final concentration in the roots was higher in the daylight group than in the fluorescent-light set.

The apparently higher overall concentration in the new growth compared with the bulked samples from the treated area for plants in daylight reflected the higher proportion of stem to leaves in the treated area, combined with the much greater accumulation of schradan in leaves than in stems (see Table 1).

In a confirmatory experiment, made a few weeks later at slightly lower temperatures, the breakdown of schradan in the new growth was found to be of the same order as that in the treated section.

TABLE 6. *Translocation after 15 days from treated leaves to new growth and roots of apple stocks under fluorescent light* (F) *and daylight* (D). (*Each value is the mean of three replicates*)

		Treated region	New growth	Roots	Leachate
% of amount impacting	F	75·8			4·9
	D	50·4			4·3
% of amount absorbed	F	78·0	20·8	1·2	—
	D	54·1	39·2	6·6	—
% of amount translocated	F	—	94·4	5·6	—
	D	—	85·3	14·7	—
Concn. of schradan equiv. as μg./g. fresh wt.	F	177	115	2·4	—
	D	67	117	7·6	—

The % breakdown in the treated region was 74 and the concentration of schradan (as μg./g. fresh wt.) was 46 for F and 17 for D.

Translocation from leaves of different ages: effects of stem ringing

This experiment was made to investigate the effect of age of leaf, the distribution of schradan, and the path followed by the translocated material. On 1 September, twelve apple stocks each 3 ft. high and with approximately thirty leaves, were divided into three groups in which the lower (*a*), middle (*b*) and upper leaves (*c*) respectively were sprayed with 0·20% solution. In each of these groups of four plants half were ringed as recorded in Table 7. The ringing was done immediately before the spraying by removing all the tissue outside the xylem in a ring $\frac{3}{8}$ in. wide. The cut was smeared with Vaseline and protected with adhesive tape. The plants were kept 14 days in the glasshouse (50–78° F.: mean daily sunshine $4\frac{1}{2}$ hr.).

Harvesting was carried out on the three leaf sections (plus their accompanying stem portions) and on the roots. The treated leaves were leached and all sections were acid digested except sections $(b+e)$ and $(c+f)$ of the *b*-sprayed plants which were extracted and partitioned (Table 7*b*).

Table 7*a* shows that the order of translocation in the unringed plants was *b*-sprayed > *a*-sprayed > *c*-sprayed. From the *a*-sprayed plants more was translocated to *c* and *h* than to *b* but the order of concentration of schradan equivalent was $c>b>h$. For the *b*-sprayed unringed plants the order of translocation was $c>h>a$, while the concentration of schradan equivalent was $c>a>h$. Proportional translocation from the *c*-sprayed unringed plants was $b > h > a$ and the concentration of schradan equivalent was $b > a > h$.

The effect of ringing depends on the zone treated. For the *a*-sprayed plants ringing almost doubled the proportion translocated and increased the concentration of schradan equivalent particularly in *c* and *h*. Ringing *b*- and *c*-sprayed plants practically eliminated downward and much reduced upward translocation. The low concentration of schradan equivalent translocated from the *b*-sprayed ringed plants to the *c* section, coupled with the increased breakdown in that section resulted in a 13-fold decrease in the concentration of schraden in *c* (Table 7*a*).

Table 7*a*. *Effect of ringing on translocation and breakdown in apple stocks 14 days after treatment of leaves of different ages*

		Sections analysed							
		a+g		*b+e*		*c+f*		*h*	
Section sprayed	Ringing treatment	% of amount absorbed	Concn. of schradan equiv. μg./g. fresh wt.	% of amount absorbed	Concn. of schradan equiv. μg./g. fresh wt.	% of amount absorbed	Concn. of schradan equiv. μg./g. fresh wt.	% of amount absorbed	Concn. of schradan equiv. μg./g. fresh wt.
a	No ringing	79·1	89	4·5	4·2	8·1	6·3	8·4	1·4
	Ringed above and below *a*	61·4	55	8·7	5·9	16·3	12·9	13·6	2·8
b	No ringing	4·3	7·1	69·2	85·0	15·5	20·0	11·0	3·0
	Ringed above and below *b*	0·3	0·3	95·4	83·0	4·4	4·8	0·0	0·0
c	No ringing	2·0	3·0	4·2	5·1	89·7	131	4·1	1·2
	Ringed below *c*	0·2	0·2	0·0	0·0	99·8	131	0·0	0·0

Table 7*b*. *Extraction efficiency, breakdown and schradan concentration in plants with section b sprayed*

	Sections analysed					
	b+e			*c+f*		
Ringing treatment	% extraction efficiency	% break-down	Concn. schradan μg./g. fresh wt.	% extraction efficiency	% break-down	Concn. schradan μg./g. fresh wt.
No ringing	94	46	47	96	49	10·4
Ringed above and below *b*	91	61	32	97	85	0·8

Ringing after spraying the *b* leaves therefore reduced translocation of schradan equivalent and increased breakdown in the treated section and also in the material translocated to the section above. The effect produced by ringing *b*- or *c*-sprayed apple plants was completely opposite to that produced by ringing comparable *a*-sprayed plants.

EXPERIMENTS ON CHRYSANTHEMUMS

Translocation from lower, middle and upper leaves

In June plants 18 in. high with eighteen to twenty leaves were sprayed in sections with a 0·2% solution, as previously described for apple (p. 507). After 7 days in the glasshouse (51–81° F.) the plants were harvested into the following sections (Fig. 1) *a*+*g*, *b*+*e*, *c*+*f* and *h*, and for the *c* treated plants the newly developed foliage above the *c* section was harvested separately. Sections extracted and partitioned were *a*, *b* and *c* for *a* treated, *b* and *c* for *b* treated and *c* for *c* treated.

TABLE 8*a*. *Translocation of schradan equivalent in chrysanthemums after 7 days following application in June to lower, middle and upper leaves respectively.* (*Each value mean of three replicates*)

	Sections analysed									
	a+*g*		*b*+*e*		*c*+*f*		New growth		*h*	
Section sprayed	% of amount absorbed	Concn. of schradan equiv. μg./g. fresh wt.	% of amount absorbed	Concn. of schradan equiv. μg./g. fresh wt.	% of amount absorbed	Concn. of schradan equiv. μg./g. fresh wt.	% of amount absorbed	Concn. of schradan equiv. μg./g. fresh wt.	% of amount absorbed	Concn. of schradan equiv. μg./g. fresh wt.
a	95·4	348	2·2	3·4	2·1	5·7	*		0·3	0·7
b	0·5	1·7	95·8	183	3·5	5·4	*		0·1	0·3
c	0·03	0·1	0·4	0·8	96·8	238	2·7	41	0·03	0·03

* Bulked with *c*+*f*.

TABLE 8*b*. *Extraction efficiency, breakdown and schradan concentration in plants of Table* 8*a*. (*Each value mean of three replicates*)

	Sections analysed								
	a+*g*			*b*+*e*			*c*+*f*		
Section sprayed	% extraction efficiency	% break-down	Concn. schradan μg./g. fresh wt.	% extraction efficiency	% break down	Concn. schradan μg./g. fresh wt.	% extraction efficiency	% break-down	Concn. schradan μg./g. fresh wt.
a	97·7	7·6	322	85·6	9·8	3·0	97·8	6·5	5·3
b	(103)	(26·6)	(1·2)	90·4	6·7	171	94·3	13·1	3·7
c	—	—	—	—	—	—	94·6	8·5	217

( ) Values in brackets based on single observation with low counts.

The results (Table 8*a*) show that translocation from all treated zones was of the same order, thus differing from the corresponding data obtained for apples (Table 2), where more was translocated from *a* and *b* treated leaves than from *c*. The distribution of translocated material followed the same pattern in both crops.

Table 8*b* shows that as in apple, the breakdown was of a similar order throughout the plant.

Effect of light on translocation

The effect of exposure to fluorescent light was studied over a period of 4 hr. to 16 days. The plants had twelve open leaves, the eight middle leaves being sprayed with a 0·5 % solution in April and then given one of the following sequences of treatment, each of which started with a light period and was carried out at 63–75° F.

a. Plants given 18 hr. light each 24 hr. S.I. (standard illumination) and harvested at 4, 8, 24, 48, 96, 192 and 384 hr. after spraying.

b. Plants given initial exposures of 1, $2\frac{2}{3}$, 4 or 8 hr. light and harvested 48 hr. after spraying.

c. Plants given initial light exposure as in (*b*) followed by 47 hr. darkness, then treatment (*a*) and harvested 96 hr. after spraying.

Each value in Tables 9*a*, *b* and *c* is the mean of three replicates.

Table 9*a* shows that whilst the concentration of schradan equivalent increased slowly over the period, the breakdown after 96, 192 and 384 hr. was low.

Table 9*b* shows that there may be a direct relationship between the period of exposure to light and the final concentration in the rest of the plant.

Table 9*c* shows that plants placed in the dark for 47 hr. when again exposed to standard illumination translocated at a rate comparable to that of plants under these conditions for the whole period.

EXPERIMENTS ON BROAD BEANS

Assessment of schradan equivalent in root excretions

A preliminary attempt to assess root excretion of ^{32}P was made on broad bean plants grown in sand. The leaves were given four applications of a 0·4 % solution and the plants were then kept 11 days in the laboratory (65–70° F.). At harvest, the sand was leached with 0·001 % non-radioactive schradan and the leachate combined with root washings, the whole being concentrated to a small volume for precision of assay. No activity was detected in any of the sand washings and less than 0·1 % of the original deposit was found in the roots.

In a second experiment the roots of six bean plants with four open leaves were carefully washed and the plants placed with their roots in nutrient solution. After 3 days the leaves were sprayed with a 0·2 % solution and then kept under fluorescent light for 18 hr. per day for 11 days (64–78° F.). The level of the nutrient solution was maintained by the addition of further amounts as required. In all but two plants the rate of uptake of nutrient solution fell off during the period because of phytotoxic effects. At harvest the roots were rinsed and the rinsings added to the nutrient solution.

Aliquots of the nutrient solution were withdrawn for radioassay on the 4th, 7th, 10th and 11th day and subsequently returned. Although a slow increase in activity of the nutrient solution was recorded throughout, the maximum for any sample was only 0·9 % of the original leaf deposit. The analysis at harvest (mean of six replicates)

TABLE 9*a*. *Translocation and breakdown in chrysanthemums given 18 hr. light in each 24 hr. (standard illumination)*

Interval (hr.) between treatment and harvest	Treated leaves			Rest of plant	
	% of amount absorbed	Concn. of schradan equiv. µg./g. fresh wt.	Breakdown	% of amount absorbed	Concn. of schradan equiv. µg./g. fresh wt.
4	98·7	207	—	1·2	1·0
8	99·1	356	—	0·9	1·3
24	98·3	373	—	1·6	3·6
48	97·9	890	—	2·1	7·9
96	96·1	904	4·7	3·9	14·9
192	94·9	691	8·6	5·1	13·5
384	91·1	1062	14·0	8·9	19·4

TABLE 9*b*. *Translocation of schradan equivalent in chrysanthemums harvested 48 hr. after spraying*

Treatment Light and Dark	Treated leaves		Rest of plant	
	% of amount absorbed	Concn. of schradan equiv. µg./g. fresh wt.	% of amount absorbed	Concn. of schradan equiv. µg./g. fresh wt.
1 L 47 D	99·5	413	0·5	0·9
2⅔ L 45⅓ D	99·3	495	0·7	1·0
4 L 44 D	99·4	344	0·6	1·0
8 L 40 D	98·9	399	1·0	1·6
48 hr. S.I.	97·9	890	2·1	7·9

TABLE 9*c*. *Translocation of schradan equivalent in chrysanthemums harvested 96 hr. after spraying*

Treatment Light and dark	Treated leaves		Rest of plant	
	% of amount absorbed	Concn. of schradan equiv. µg./g. fresh wt.	% of amount absorbed	Concn. of schradan equiv. µg./g. fresh wt.
1 L 47 D and *N to 96	98·5	709	1·5	5·1
2⅔ L 47 D and *N to 96	98·1	794	1·9	6·8
4 L 47 D and *N to 96	98·0	752	2·0	6·0
8 L 47 D and *N to 96	97·9	737	2·1	6·9
Standard illumination (*N) control	96·1	904	3·9	14·9

*N = Standard illumination.

showed that of the original deposit 86·1 % was in the leaves and stem, 2·9 % in the roots and 0·6 % in the nutrient solution. In view of the rapid degradation occurring in beans (Table 10) it appears that the small amount of schradan excreted by the roots can be neglected.

Translocation from leaves to new growth and roots over 28 days

Thirteen bean plants receiving approximately 9 hr. daylight daily were sprayed with 0·3 or 0·05 % solution and kept at 80° F. Plants were harvested after 3, 6, 14 and 28 days, and assayed in three samples: (i) treated leaves, (ii) new growth plus stems of the treated section, (iii) roots and lower stem. Breakdown was determined in samples (i) and (ii).

TABLE 10. *Translocation and breakdown in broad beans at* 80° F. *in daylight.* (*Mean of two replicates*)

Concn. of solution (%)	Interval between treatment and harvest (days)	Treated leaves		New growth and stem of treated section		Roots and lower stem
		% of amount absorbed	% breakdown	% of amount absorbed	% breakdown	% of amount absorbed
0·3	3	97·3	12	2·8	—	*
0·3	6	96·8	26	2·9	62	0·35
0·05	6	92·0	46	7·9	70	0·11
0·3	14	91·9	41	7·1	82	1·49
0·05	14	89·1	47	9·7	72	1·22
0·3	28	88·6	70	11·4	90	*

* Included with new growth and stem of treated section.

The breakdown of the material in the treated leaves (Table 10) was lower than that elsewhere. As the increase in the percentage translocated (2·8 after 3 days to 11·4 after 28 days) was accompanied by a corresponding increase in breakdown, over this period approximately 1 % of the absorbed material was present as schradan elsewhere in the plant, but because of new growth the concentration of schradan fell continuously. Translocation in plants treated with 0·05 % solution was greater after 6 days than in plants treated with 0·3 % solution, but this difference was less marked after 14 days (cf. Table 5).

Light and temperature effects over 3 days

Eight broad bean plants, sprayed with 0·3 % solution were kept at controlled temperatures in daylight and darkness as described in Part I. At harvest, 72 hr. after treatment, the treated leaves were leached and the rest of the plant, including the roots, bulked as a separate sample. Breakdown of schradan in the leachate and treated leaves was determined, and a schradan equivalent value obtained for the translocated material (Table 11).

All the proportions translocated were low, but light appeared to have more effect than heat in promoting translocation and in inducing breakdown. In darkness, however, there was more translocation at 80° than at 50–60°.

An example of breakdown in the leachate has been previously discussed in Part II and the explanation of the figures given in Table 11 is probably similar. The radiochemical purity of the solution was 99 %, so preferential absorption of schradan could give rise to breakdown data in the leachates of the order shown. In some of the plants there is also the possibility of additional slight breakdown in the cuticular layer.

TABLE 11. *Translocation and breakdown in broad beans after 3 days under different temperature and light treatments. (Mean of two replicates)*

	Leachate		Treated leaves		
Treatment	% of amount deposited	% breakdown	% of amount absorbed	% breakdown	Translocated as % of absorbed
Daylight: 80° F.	4·5	10·6	97·3	12·2	2·7
Daylight: 50–60° F.	11·8	8·4	97·5	12·4	2·5
Dark: 80° F.	12·5	3·6	99·1	9·6	1·0
Dark: 50–60° F.	28·2	10·5	99·9	9·7	0·1

EXPERIMENTS ON *COLEUS*

Effect of source of light

Translocation in July was determined in five plants given a 0·27 % solution spray on their middle leaves. Two of the plants were kept in the glasshouse and three under fluorescent light for 18 hr. daily (63–85° F.). After 7 days the treated leaves were leached, and the remainder of the plant including the roots, was acid digested. The data, Table 12, show that there was no difference in the effects of the two light treatments.

TABLE 12. *Translocation of schradan equivalent in* Coleus *after 7 days in July*

	Treated leaves				Rest of plant	
	% of initial deposit					
Light treatment	In leachate	Absorbed	% of amount absorbed	Schradan equiv. in μg./g. fresh wt.	% of amount absorbed	Schradan equiv. in μg./g. fresh wt.
Daylight	17·1	69·4	98·0	198	2·1	1·8
Fluorescent	18·2	61·7	96·4	144	3·6	1·5

Behaviour of different plant species under the same experimental conditions

Coleus *and beans*

A preliminary experiment with *Coleus* in February showed that plants given a foliage spray of 0·3 % solution and kept 6 days in daylight at 80° F. showed a slightly higher translocation rate and a much smaller breakdown figure (8 % in treated leaves, 10% in new growth and stem of treated section) than the comparable broad bean plants recorded in Table 10.

In a further experiment in February, the leaves of broad beans and *Coleus* were sprayed with a 0·2% solution and the plants placed in the laboratory (60–79° F.) for 4 days.

Table 13 shows that translocation was slightly more in *Coleus* than in beans and that the breakdown was very much less, confirming preliminary results. In both species the breakdown was greater in translocated material (cf. Table 10 for beans).

Table 13. *Translocation and breakdown in broad beans and* Coleus *after 4 days in daylight in February. (Mean of three replicates)*

	Treated leaves			Rest of plant excluding roots		
Plant species	% of amount absorbed	Extraction efficiency	% breakdown	% of amount absorbed	Extraction efficiency	% breakdown
Bean	95·4	100	11·4	4·6	95·6	33·7
Coleus	93·1	97	0·5	6·9	86·1	3·7

Table. 14. *Translocation and breakdown of schradan in* Coleus, *broad beans and runner beans under different light conditions after 72 hr. (Each value is mean of two replicates)*

	% of amount absorbed in rest of plant		
Treatment	*Coleus*	Broad beans	Runner beans
Light to Light	7·3 (1·5, 1·7)	5·6 (11·3, 20·7)	3·4
Dark to Light	2·9	2·5	4·3
Light to Dark	6·0	5·4	0·06
Dark to Dark	0·9 (0·3, 0·0)	0·7 (5·1, 41*)	0·3

* Values based on very low count.

( ) Values in brackets following the entry refer to breakdown in the treated leaves and the rest of plant including roots, respectively.

Effect of different pre- and post-light treatments

The effect of light immediately preceding and following treatment was studied with *Coleus*, broad beans and runner beans. For 36 hr. before spraying, half the plants of each species were given fluorescent light for 18 hr. each day and the other half kept in the dark. After spraying with a 0·5% solution, each of these two sets of plants were divided into two groups, one maintained under the same light conditions and the other in darkness for the 72 hr. (60–70° F.).

Table 14 shows that for *Coleus* and broad beans light before treatment was most important, while for runner beans translocation was reduced by the absence of light after treatment.

In *Coleus* and broad beans breakdown was enhanced by light.

In another comparative experiment on these crops treated in January with a 0·5% solution, some were placed in daylight in the laboratory (64–77°) and some under standard fluorescent (66–76° F.). The results on translocation confirmed those already given.

Table 15 shows that the breakdown in broad beans in daylight was comparable to that in runner beans under fluorescent light, whilst that of *Coleus* was considerably less, and this was reflected in the higher concentration of schradan in *Coleus*.

TABLE 15. *Breakdown and concentration of schradan in the treated and untreated parts of* Coleus (K), *broad beans* (B) *and runner beans* (R) *7 days after treatment*

		Treated leaves			Rest of plant		
Species	Light treatment	% of amount absorbed	% breakdown	Schradan as μg./g. fresh wt.	% of amount absorbed	% breakdown	Schradan as μg./g. fresh wt.
K	Standard	91·2	4	605	8·8	7	29
B	Daylight	91·1	40	379	8·9	86	2
R	Standard	92·5	38	570	7·5	65	17

Relation of concentration of schradan to insecticidal activity

To relate insecticidal activity to the data obtained in these experiments, values for threshold toxic concentrations of schradan in plant tissue have been determined for the following species of aphids:

Black bean aphid	*Aphis fabae* Scop.	10–15 μg./g. fresh wt. of plant tissue
Chrysanthemum aphid	*Macrosiphoniella sanborni* (Gill.)	20–25 μg./g. fresh wt. of plant tissue
Green apple aphid	*Aphis pomi* Deg.	20–30 μg./g. fresh wt. of plant tissue

These values were determined by treating mid-section leaves of plants with the labelled schradan and infesting the sections above some of them with a mixed population of aphids. When approximately 80% kill had been obtained, the corresponding sections of uninfested plants were radioassayed for schradan content. The concentrations given are mean values for a section; the values at any point depend on the distribution within the tissues, and this may not be uniform.

DISCUSSION

The efficiency of schradan as a systemic insecticide is dependent on its translocation to other parts of the plant, and on the rate and extent of its breakdown into non-insecticidal metabolites. Schradan itself is a weak cholinesterase inhibitor but within certain tissues it is converted to an active metabolite, believed to be the toxic agent. Plant extracts from bean (Hall, Stohlman & Schechter, 1951) and lettuce (Dubois, Doull & Coon, 1950), treated with schradan, have been shown to be cholinesterase inhibitors. Hartley (1951), however, suggests that the concentration of toxic derivative in the plant is always low. Duspiva (1951) and O'Brien & Spencer (1953) have shown that the insect is capable of producing the active metabolite, and the latter workers suggest that the susceptibility of insects to schradan is dependent on its successful penetration into the nerve cord before its decomposition has occurred. Our results have been expressed in terms of two quantities only—'schradan' and 'schradan equivalent'. The former refers to undecomposed schradan as determined by chloroform/aqueous alkali partition, and the latter is a calculated value assuming that all the ^{32}P is present in the chemical form of schradan.

In the experiments described the most important single factor in promoting translocation would appear to be light, and the fluorescent light used was apparently no less effective than daylight. In an experiment on *Coleus*, broad beans and runner beans, very little translocation occurred if the plants were kept in the dark before and after treatment. The effect of light is not, however, a simple one, and interesting differences in the behaviour of the plant species have been observed. Thus with runner beans efficient translocation requires the presence of light, whilst for broad beans and *Coleus* it occurs in the dark provided the leaves have been exposed to light before treatment (Table 14). The importance of light and photosynthate concentration of leaves for the toxicity of 2, 4-D has been shown for Red Kidney beans (Davis & Smith, 1950) and for Snap beans (Mitchell & Brown, 1946). The effect of light on the translocation of schradan is probably not direct, but dependent on the fact that in some plants active photosynthesis and in others the products of this process, are necessary for its movement.

By ringing apple stocks above and below the treated leaves it has been shown that phloem is the main conducting tissue involved in the transport of schradan and its metabolites from the middle and top leaves. Whilst downward translocation from these regions would appear to take place almost exclusively in the phloem, limited upward movement takes place in xylem. The increased translocation which occurs in ringed plants with the lowest leaves treated is difficult to explain. Wedding (1953) found that following application of schradan to bean and lemon leaves the first movement was in phloem, but that after a time diffusion into, and subsequent transport in xylem does occur to a limited extent. He also suggested that temperature may be an important factor in promoting the transport of organic molecules in phloem. Some evidence has been obtained with broad bean (Table 11) that a higher temperature may in fact increase translocation of 'schradan equivalent' in the dark.

No great differences in the proportion of translocated to absorbed have been found between beans, *Coleus* and chrysanthemums, in all of which the proportion of translocated to absorbed is about 1% per day. Values of up to 4% per day have, however, been observed with apples. Metcalf & March (1952) found that between 0·1 and 1·0% of the total dosage of schradan applied to a single lemon leaf appeared in the other leaves after 17 days. David (1951) stated that after repeated application to the six lower leaves of broad beans the concentration in the leaves above was 5%, and in the stem apex 10%, of that remaining after 8 days in the treated leaves. If, however, it is assumed that the weights of the untreated leaf and stem apex are both of the same order as each of the treated leaves, the data would correspond with the translocation in 8 days of only 1 % of the applied material. Zeid & Cutcomp (1951), using a bioassay technique, concluded that most translocation occurred in actively growing plants, and have quoted a value of 40 % for the extent of translocation of schradan or an active toxin into unsprayed leaves 12 days after spraying. As the active metabolite is much more toxic than schradan it is possible that small quantities of this material were giving a misleading impression of the proportion of material translocated.

The distribution of translocated material, both in amounts and direction, varies according to the foliage zone treated. In general the movement of the material is towards the new growth. Thus, if the lowest leaves of apples and chrysanthemums are treated, approximately equal proportions are found in the two leaf regions above, but the younger leaves at the top usually have a higher concentration due to their smaller weight, as observed by David (1951). Where middle-section leaves are treated translocation to the young leaves above is always greater than that to the older leaves below. Definite evidence has, however, been obtained that undecomposed schradan does travel downwards from mid-section leaves to the lowest leaves in apples (Table 5) and in chrysanthemums (Table 8*b*) though probably the amounts involved are not of insecticidal consequence. Zeid & Cutcomp (1951), using bioassay methods, claim that ten times as much of the insecticidal ingredient appeared in the leaves below those treated than in those above.

Concentrations necessary to kill certain species of aphids have been determined. These values are somewhat lower than those quoted by David (1951) for *Aphis fabae* on bean.

Further subdivisions of the plant have shown that quite high local concentrations exist within a region particularly in the new apical growth. Even if such smaller sections of the plant are taken for analysis, the concentration within the phloem tissue on which the aphides feed is much more difficult to assess and estimates of the schradan concentration necessary for aphid control can be only tentative. It has been noted with apples, the only woody plant used, that quite high proportions are translocated to the lower part of the stem and roots, particularly late in the season, and this may well be associated with storage.

The breakdown of schradan into non-chloroform extractables has been found to vary widely between the species as has been previously reported (Bennett & Thomas, 1953). Whilst in our experiments there is some indication (Table 11) that breakdown is somewhat increased by light, no great variability has been found in its value within a species at different times of the year but the conditions under which our experiments were carried out did not simulate those in the field. The long-term experiment late in the season on apples (Table 4) showed no increase in breakdown between 39 and 60 days and would confirm the observations of Heath & Llewellyn (1953) that breakdown varies with the season and is probably associated with the slowing down of metabolic processes. Metcalf & March (1952) concluded that only a small fraction of the absorbed schradan in lemon seedlings was metabolized to non-chloroform extractables by the plant in 46 days. From their data the distribution coefficient (chloroform/alkali) of the plant extract remained reasonably constant, but as continuous and increasing root absorption of schradan occurred over the period their data are difficult to interpret.

Ripper, Greenslade & Hartley (1951) and Heath (1953) have stated that root losses of schradan metabolites occurred from strawberries. Such losses were suggested by Heath to account for the different breakdowns observed in plant species. Park & Heath (1952), have, however, since stated that root excretion is always slight but that guttation may account for losses in some crops. Little to no loss by excretion from roots of broad beans was observed in our experiments.

It has been shown that in some plants such as apples and chrysanthemums the breakdown is of the same order throughout the plant, whilst possibly in *Coleus* and especially in broad beans the breakdown of the material translocated to the untreated zones is greater. A higher observed breakdown in the untreated regions could arise in several ways. Thus breakdown may proceed at the same rate in all parts of the plant but breakdown products might move preferentially to schradan from the treated leaves. Alternatively if the breakdown rates vary in different parts of the plant, the results obtained could be explained whatever the relative translocation rates of schradan and its metabolites were.

Evidence from some experiments would suggest that a higher efficiency of translocation occurs from lower concentrations within the treated section, but this is not borne out in all experiments.

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THE BIOLOGY AND CONTROL OF *OTIORRHYNCHUS CLAVIPES* BONSD. (RHYN. COLEOP.), A PEST OF STRAWBERRIES

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(With Plate 16 and 5 Text-figures)

Otiorrhynchus clavipes Bonsd. is a serious pest of strawberries around Cheddar, Somerset. The weevils nibble the foliage, the larvae eat the roots of the plants, and burrow into the rootstock.

The beetles appear in two waves, pupating in the autumn and emerging *en masse* in the spring, or pupating in the late spring and summer and emerging in succession between mid-June and the end of August. Both sexes are found and can be distinguished by the posterior thoracic sterna, which are concave in males and convex in females. Mating is alternated with egg-laying and the beetles lay between 100 and 300 eggs per female. On emergence the beetles eat voraciously, and roughly one egg is laid for every 10 sq.mm. of leaf consumed. Both parthenogenetic and fertile eggs are laid and hatch in between 17 and 24 days.

The larvae burrow rapidly when hatched and can only penetrate soil spaces which will admit their head capsules. The distribution of head capsule widths in mixed populations of larvae of all ages shows there are five instars. While feeding, the larvae are clustered within the root range of the plants and find their way to their food from plant to plant, guided by the exudates from the strawberry roots. As the larvae grow older they descend in the soil and turn to white soft pupae in smooth earthen cells 6–8 in. below the plants.

Adult damage to foliage is unimportant but larval damage to roots is serious and has been largely responsible for restricting the effective life of the strawberry beds to at most 2 years. Although weevil attack can be avoided by growing the crop in a 1-year rotation, control is difficult owing to the intensive cropping, which creates the most suitable conditions for the spread of the infestations. The adults are killed with 10% DDT dust; and the larvae by DDT and BHC preparations poured on to the soil, at a pint per plant, or by DD injection. The limitations and possibilities of various methods of control are discussed.

Introduction

Otiorrhynchus clavipes Bonsd. (syn. *Brachyrrhynus tenebricosus* Lat., *Otiorrhynchus tenebricosus* Herbst.), is usually described as a pest of top and bush fruit and only occasionally attacks strawberries in this country (Miles, 1921; Massee, 1946). Nevertheless, at Cheddar, Somerset, *O. clavipes* is responsible for much damage to the strawberry crop each year. Apart from isolated individuals, which are usually *O. sulcatus* Fabr. or *O. ovatus* L., it is the only species of *Otiorrhynchus* to be found in the area. As with other species of *Otiorrhynchus* on strawberries (Fidler, 1936), it is the larvae of *O. clavipes* which cause most damage, while on top and bush fruit the adults are the more serious pest.

The adult beetles found at Cheddar have been identified as *O. clavipes* var. *fuscipes* Oliv. by the British Museum (Balfour-Browne *in litt.*) and as *O. clavipes* subsp. *lugdunensis* Boh. (Hoffmann, 1948, and *in litt.*), although both the British Museum and Massee (*in litt.*) suggest that *O. clavipes* is simply a very variable species. The life history of the Cheddar beetles differs appreciably from that described for *O. lugdunensis* by Hoffmann and from that described for *O. clavipes* generally by Massee. It also differs from the general pattern of life history common to other *Otiorrhynchus* spp. (Fidler, 1936) attacking strawberries, and control measures which are suitable for other strawberry weevils are not necessarily applicable to the Cheddar populations.

Most of the strawberry crops at Cheddar are grown in a 2-year rotation. Runners are planted in July and August, the plants are cropped in June in the two following years, and are ploughed up as soon as possible after the second crop has been picked. The life history of *O. clavipes* is closely integrated with this programme, and investigations into the biology and control of the weevils are still in progress. A preliminary account of work to date and the significance of the observations in relation to control measures is given below.

DAMAGE

Strawberries

The beetles damage the plants, in the same way as other strawberry weevils, by clasping the edge of the leaf and eating away semicircular areas (Pl. 16*a*). The strawberry plant puts out from the crown a continual succession of leaves during the year. These are at first erect in the centre of the plant and as they grow older bend down to ground level and are pushed to the outside of the plant. Even when weevil bites are distributed over the whole plant, those on the maturing leaves are usually fresh, while those on the older, mature or senescent leaves have callus on the cut edges indicating their age. Nevertheless the beetles ate young and old leaves indiscriminately, when offered both together in the laboratory, and it is unlikely that they have an actual preference for young leaves in the field. A more probable explanation is that the beetles tend to climb to the highest points on the plants before beginning to feed, and this automatically brings them to attack young leaves rather than old ones.

The effect of leaf damage on the yield of fruit is unimportant, as the damage is at a peak in spring, when the plant is replacing its leaves most rapidly.

The larvae of *O. clavipes* eat the roots of the strawberry plant and this causes serious loss of yield. They always attack the feeding roots of the current season, and even when these have been completely bitten off, the old roots of the previous year's growth are still left undamaged (Pl. 16*b*). Attacked plants either become prematurely senescent in the autumn so that the leaves go red, or they suddenly wilt, often without warning in May and June, just when the fruit is forming; at other times they appear unthrifty and slightly stunted. Other causes of stunting such as mites, eelworms, or infection by virus diseases leave the root system intact, and damage due to weevil attack can be distinguished by the ease with which the plants can be pulled up.

If maiden plants are attacked they seldom show signs of damage before their first winter, but usually wilt before fruiting. Second-year plants can show signs of damage at either end of the year, but most frequently fail in the autumn following the maiden crop. Plants older still are usually free from infestation, otherwise, under current practice, they would have been ploughed up after the second crop. The importance of larval damage can be estimated by the fact that most growers have found it uneconomic to crop their strawberries for more than 1 or 2 years.

Weeds

Since weeds in the strawberry fields often show bites which look as though they might have been made by weevils, starved weevils were kept in tubes containing fresh weeds common around Cheddar. Feeding readily took place on scentless mayweed (*Matricaria inodora* L.), mouse-ear chickweed (*Cerastium vulgatum* L.) and sowthistle (*Sonchus oleraceus* L.), but goosefoot (*Chenopodium album* L.), sun spurge (*Euphorbia helioscopia* L.) groundsel (*Senecio vulgaris* L.), plantain (*Plantago* spp.), clover (*Trifolium pratense* L.) common orache (*Atriplex patula* L.), dandelion (*Taraxacum officinale* Weber), and buttercup (*Ranunclus repens* L.) showed no signs of damage even after the beetles had been starved for several weeks. In similar experiments with anemone, potato, spring cabbage, and lettuce, which are the common alternative crops to strawberries in Cheddar, there were no signs of feeding.

Larvae have never been found under weeds in the strawberry beds and there has been no suggestion that they normally eat the roots either of weeds or of any of the alternative crops to strawberries usually planted in Cheddar.

LIFE HISTORY AND BEHAVIOUR

The adults

The adults (see Joy, 1932) are black, red-legged, wingless weevils, approx. 8–12 mm. long, with ovate abdomen, short stout rostrum, and elbowed antennae (Pl. 16*c* and *d*). On emerging from their pupal cells they are soft and brown, only becoming hard and black after exposure to the air for several days. They are nocturnal, hiding in the upper layers of loose soil and in the plant crowns by day, and emerging to feed on the leaves at night.

Both sexes are found, on the average in equal proportions, and males can usually be distinguished by a thinner and more pointed abdomen. There are many intermediate shapes, however, and for practical purposes the curvature of the posterior thoracic sternite has been the best index of sex (Pl. 16*e*). This is slightly convex in females and obviously concave in males, a character which appears to be well defined.

Individual weevils are found in February and March, but in the first few days of April they suddenly appear in large numbers. The first mass appearance on the observation site occurred in 1951 on 3 April, in 1952 on 6 April and in 1953 on 26 March. On these occasions several thousand beetles may appear on a few rows of plants in the course of 2 or 3 days, particularly after a spell of fine weather. Numbers reach a maximum within 3 weeks of the first mass emergence, decrease slowly until June and early July, and then increase again and are maintained, until the colder weather in September and October. The number of beetles per plant varies from as many as eleven or twelve in heavy spring infestations, to one or two in the summer. By November they have almost disappeared and intensive search of typical overwintering quarters such as hedges, walls, and brushwood, discloses no more than isolated individuals.

The spring beetles do not appear to originate in quite the same way as those which appear during summer and autumn. In the spring until mid-April, all the beetles are soft as though newly emerged from the soil, while from mid-April to May, they are all hard as though emergence has temporarily ceased. The soft beetles cannot have pupated immediately before emergence, as pupae do not appear anywhere in the beds at this time of the year. These beetles are usually found either under the leaves of the alternative crop planted where infested beds have been ploughed up the year before, or on the rows of otherwise undamaged plants nearest such beds. This suggests that the larvae pupate in the old bed the previous autumn, remain underground in their pupal cells until the spring, and then emerge to move *en masse* to the nearest strawberry beds. The method of overwintering was confirmed when a portion of a badly infested bed was left into its third year for observation purposes, and as many as three or four beetles per plant were dug from their pupal cells beneath the plants in February.

Such beetles are usually found in the winter following a damaged second year or older crop. They are found only during the winter immediately after planting if the runners are planted in ground which already contains larvae from a previous crop, and only during the winter after a maiden crop, if the runners were infested with egg-laying beetles as soon as planted.

Between May and August most of the beetles are hard, but there is always a small proportion of soft ones. Unlike those found in the spring, these soft beetles are associated with pupae which occur during the whole of this period, and pupation and emergence seem to occur more or less continuously. After August only hard beetles are to be found, as though emergence has ceased once more. There are still numbers of pupae in the ground where second or third year strawberries were growing but these give rise to beetles which remain *in situ*. Beetles which die above ground are not replaced, so that the free populations die away and eventually disappear altogether. The growers are responsible for a good deal of mortality by killing the beetles when the beds are cultivated, and by hand-picking by torchlight at night; other beetles wander away from the beds; natural predators appear to kill some; but not all the reasons for the pronounced changes in population have been investigated.

Eggs and oviposition

The eggs are 0·6 mm. in diameter, spherical, pearly white when laid, and if viable turn black and shiny within 24 hr. Eggs laid in both field and laboratory hatched in the laboratory between 17 and 24 days at room temperature. This hatching period may well have been longer in the field owing to the lower temperatures there, and seems to be peculiar to the Cheddar beetles, since the hatching period for *Otiorrhynchus* generally, including *O. clavipes*, is given by Hoffman (1948) as 8–15 days. The eggs all hatched if kept either on a moist surface such as damp plaster of Paris, or in an enclosed space such as a solid watch-glass with lid. A large proportion survived, once they had gone black, even if completely exposed to the air. The eggs from the field were slightly larger than those from the laboratory but there seemed to be no difference in viability.

In the field the eggs are only to be found scattered through the upper 1 cm. of loose soil beneath the plants. In the laboratory, beetles laid eggs freely when caged on strawberry leaves in water under upturned jam jars, or on potted plants under glass cylinders closed with muslin. The eggs were either deposited in the upper 1 cm. of loose soil in the pots, squeezed between the jar and its platform, poked through the muslin roof, or even laid on the surfaces of the leaves or glass.

The first eggs were not found in the field in 1952 until 6 June, but this was probably due to the unsatisfactory method of sampling then in use, for in 1953 they were first found on 21 May. In both these years eggs were collected until the end of August; they were most readily obtained from soil samples by floating them off in strong brine, the specific gravity of which is slightly greater than that of the eggs. They float just beneath the surface and if the scum is drawn to one side with the edge of a piece of paper, they float out from beneath it and can be picked out.

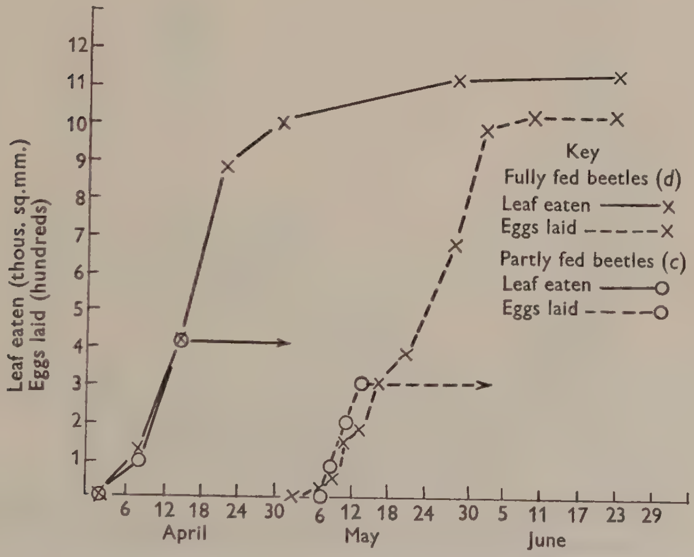
Mating occurs in the field about 3 weeks after the first spring emergence and also in July and August. In the laboratory, periods of egg-laying alternated with periods of mating. Although according to Hoffmann *O. clavipes* race *lugdunensis* lays thirty-two to fifty-five eggs, caged female *O. clavipes* from Cheddar laid between one-hundred and five-hundred eggs each (Table 1). Parthenogenetic eggs were indistinguishable from fertilized eggs in number, size, and viability. In the field, light infestations by larvae and wandering fecund females occasionally occurred at considerable distances from any previous infestation, and it seems likely that parthenogenesis plays an important part in the spread of the attacks.

If dug from their pupal cells in winter, females caged on plants out of doors immediately burrowed into the soil again, while if caged on plants in the laboratory, they laid fertilized or parthenogentic eggs quite normally.

Beetles emerging in the field ate voraciously, and apparently continuously at night for the first week or 10 days after emergence. After this, new leaf damage on the beds became less abundant, and on any given night even in the most suitable weather, only a few beetles were found on the plants. A possible relationship between feeding and oviposition was illustrated in the laboratory. Isolated soft beetles from the first spring emergence were caged on strawberry leaves and allowed to feed for different periods of time. The leaves were replaced when they were either half eaten or had lost their freshness. Regular egg counts were made, and the amount of leaf eaten was measured periodically in sq.mm. (Text-fig. 1).

TABLE 1. *Number of eggs laid by isolated female* O. clavipes (*five beetles per treatment*) *taken from the field while still soft immediately after emergence and caged under 2-lb. jam jars on fresh strawberry leaves in water. In treatment (e) only, each female was accompanied by a male in the same jar*

	No food (*a*)	Fed 5 days 2–7 April (*b*)	Fed 14 days 2–16 April (*c*)	Fully fed (*d*)	Fully fed and mated (*e*)
	0	0	5	136	199
	0	0	48	386	153
	0	0	65	110	224
	0	0	137	168	175
	0	0	50	226	430
Total	0	0	305	1026	1181

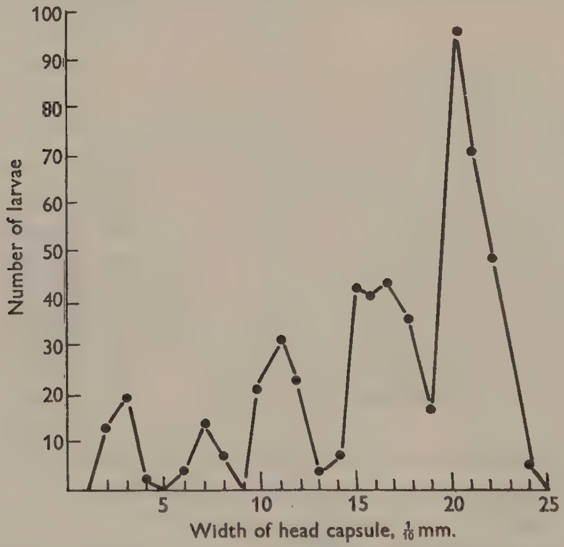


Text-figure 1. Periodical running totals of eggs laid and leaf area eaten in sq.mm. by the female *O. clavipes* in treatments (*c*) and (*d*) in Table 1. (See Table 1 for further data.)

The results showed that 10 sq.mm of leaf consumed could be roughly equated with one egg, and that when deprived of food the females laid proportionately fewer eggs. Egg-laying did not begin until the rate of leaf consumption decreased and feeding became intermittent, about a month after emergence, and was completed in a further month. According to these results egg-laying could be expected to occur in the field in May, a month to 6 weeks after the first spring emergence, and the experiment substantiated the 1953 results.

Larvae and pupation

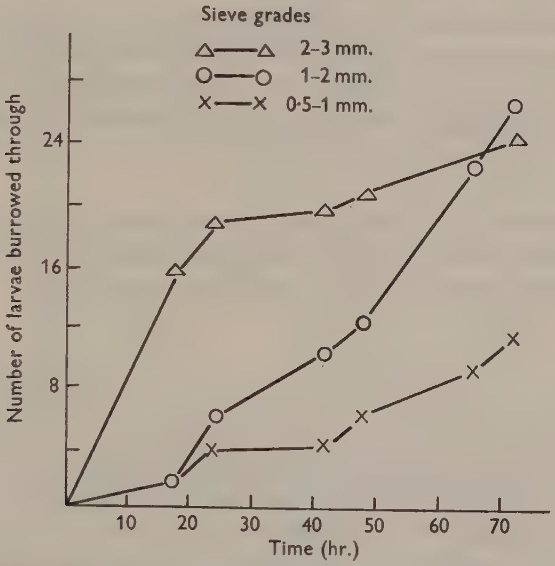
The grub is a typical apodous weevil larva, creamy white in colour, but sometimes brown or pink according to the colour of the contents of the gut. On a flat surface it crawls at considerable speed, but it is more often found at rest in a characteristic crescent-shaped curve. The size of the older larvae varies considerably from place to place and cannot be used as an indication of age. The distribution of the head capsule widths in a heterogeneous population is, however, sufficiently discontinuous to be used as an index of instar (Wigglesworth, 1947). The amalgamated distribution of head capsule widths of several batches of larvae brought in from the field at various times showed (Text-fig. 2) that there are five instars with quite well-defined head measurements.



Text-fig. 2. Amalgamated distribution of head capsule widths of four batches of *O. clavipes* larvae of mixed ages brought in from the field.

The larva eats its way out of the eggshell leaving a round hole with a ragged edge. When newly hatched it is 1–1·8 mm. long with long setae and a brown head capsule 0·2–0·3 mm. wide. It is extremely active and burrows into loose soil with great speed becoming desiccated and moribund or even dead within a few hours of hatching if completely exposed to laboratory conditions of temperature and humidity.

In the field young larvae are usually found scattered in the soil round the crown of the plant. A preliminary investigation of burrowing was made in the laboratory by scattering newly hatched larvae on the surface of cylinders of soil 1 ft. deep and 3 in. in diameter. The soil in these cylinders was sieved into three different grades, 0·5–1·0, 1·0–2·0, and 2·0–3·0 mm., all from the same soil sample, and after some time the larvae all emerged from the bottom of the cylinders containing the two coarse fractions.



Text-fig. 3. Periodical running totals of newly hatched *O. clavipes* larvae (out of twenty-six in each cylinder) burrowed through 1 ft. cylinders of soil sieved into different grades.

Text-fig. 3 gives the number of larvae that appeared after different periods of time. The rate of downward movement through the fine fraction was so slow that many of them had not appeared even after 3 days, while 75 % of them had burrowed right through the cylinder containing the coarse fraction within the first 12 hr. The spaces between particles of the latter size when spherical and closely packed are of the same order of size as the head capsule of a newly hatched larva, and penetration of the soil at first probably depends on the existence of soil spaces large enough to admit its head. This may account for the relative persistence of infestations on loose brashy soils, by comparison with the finer and more consolidated clays; more larvae would be expected to succumb to either starvation or desiccation if their penetration below the dry surface layer of soil was delayed.

The existence of a further important response which aided food-finding was illustrated by experiments similar to those above, where the larvae burrowed through coarse fraction soil (2–3 mm.) in cylinders in which either a layer of crushed strawberry roots, or alternatively a layer of string, was buried 6 in. below the surface. Although the larvae appeared within 12 hr. at the bottom of the cylinder containing string, they failed to appear at the bottom of the cylinder containing strawberry roots, and were found distributed amongst the roots themselves when the experiment was finally dismantled after a further 12 hr. If gravity were the only stimulus to which they responded, they would continue to burrow until beyond the root range of the plants, but their downward movement was evidently interrupted by a stimulus provided by the roots themselves.

The older larvae successively attacked the smaller roots, larger roots and finally even burrowed into the root-stock (Pl. 16*b*). They remained clustered more or less round the crown of the plant while feeding. In badly attacked beds, the largest numbers of even 4th and 5th instar larvae per plant were sometimes to be found beneath the undamaged plants (Table 2).

TABLE 2. *Distribution of the dead, damaged, and undamaged plants in a single row of seventy-three plants in a strawberry bed infested with* O. clavipes *larvae, according to the number of larvae found beneath each plant*

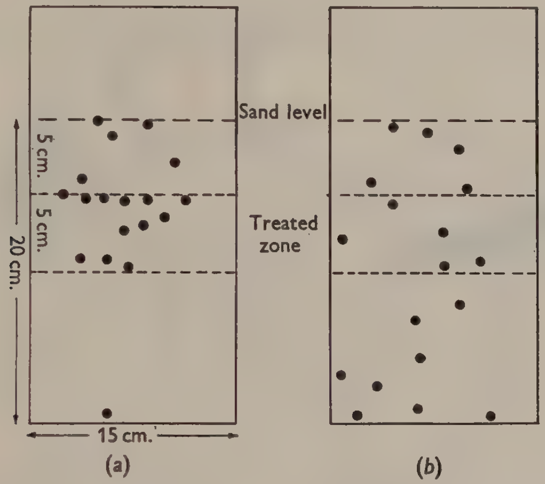
Larvae per plant...	0	1	2	3	4	5	6	7	8	9	10	11	12
Plants: Dead	3	4	5	2	.	.	.	.	.	.	.	.	.
Damaged	.	1	10	10	5	2	4	3	.	.	.	.	.
Undamaged	9	2	1	4	.	.	2	.	4	.	1	.	1

As these larvae were nearly fully grown they must have moved since they last fed, or the plant under which they were found would have shown signs of damage. Their behaviour in burrowing and root-finding was similar to that of newly hatched larvae. 4th and 5th instar larvae were made to burrow through damp sand held between glass plates just far enough apart to admit their passage. The plates supported alternate layers of damp sand, sand moistened with the filtrate from strawberry roots crushed in water, and damp sand again, all with the same moisture content.

Larvae burrowing overnight were arrested by the layer containing root filtrate (Text-fig. 4) and in similar experiments, by a layer moistened with the leachate from other sand in which strawberries had been growing.

The behaviour of wireworms attacking germinating wheat is in part due to their response to root exudates (Thorpe, Crombie, Hill & Darrah, 1945) and it seems likely that the behaviour of *O. clavipes* grubs in the strawberry beds is due to a similar response at least as far as food-finding is concerned.

As the larvae mature, they move deeper into the soil, where they eventually pupate in smooth earthen cells at 6–8 in. below the plants, giving rise to white soft pupae which occur more or less continuously from May to November; pupae found in the summer giving rise to summer beetles, and those found in autumn giving rise to spring beetles. Larvae collected in their 4th and 5th instars in winter and kept with strawberry roots between layers of damp sacking, pupated early in April and the beetles emerged in early May. The pupal period varies from 3 to 6 weeks, but in the field in summer it may well be shorter, as the temperature of the ground is then considerably higher than that of the sack cultures which were kept in a cold garage.



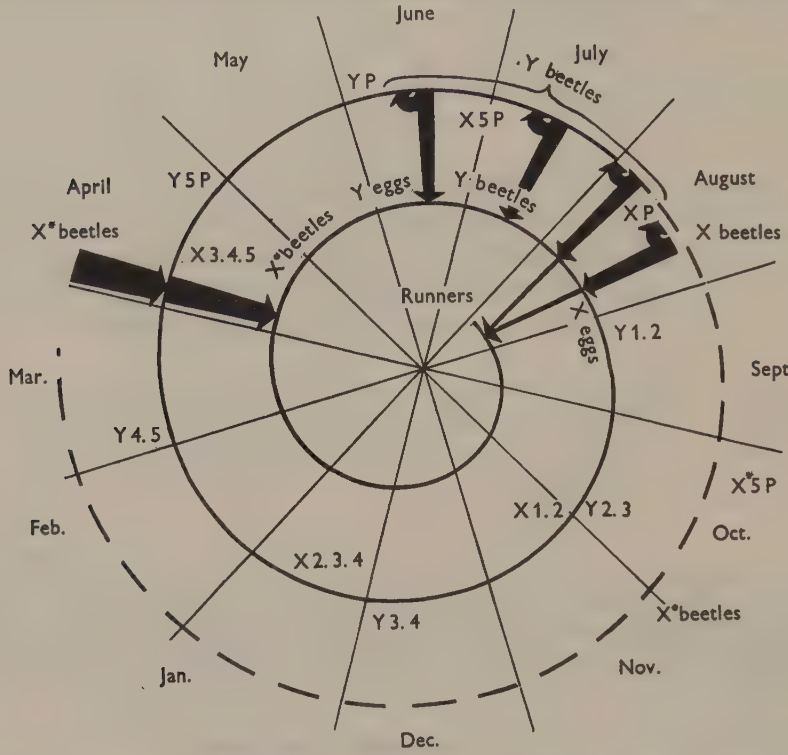
Text-fig. 4. Distribution of 4th and 5th instar *O. clavipes* larvae after burrowing overnight through sand in which a median layer had been moistened with the filtrate from water in which strawberry roots had been crushed. The diagram summarizes the results of three separate experiments each with six larvae; (*a*) treated sand, (*b*) control with clean water throughout.

Life cycle and cropping programme

The life history of *O. clavipes* in relation to the life of the strawberry plants at Cheddar is summarized in Text-fig. 5, the spiral line representing the life of the plant through two seasons of growth, with letters and figures for successive generations of beetles, grubs, pupae and eggs. Starting with runners at the centre of the diagram, the spiral is continuous to the end of the second year, representing the growing plant and is then continued as a broken line for a further 9 months, during which plant debris and beetles remain in the soil after the crop has been ploughed up.

The young strawberry plants are usually infested first by spring beetles 7–8 months after planting (wide arrow). These beetles come from old infestations in the second year or older beds, which have usually been ploughed up in the previous summer or autumn, and are called 'X*' beetles in the figure. These beetles lay 'Y' eggs in May and June which hatch in June and July. They give rise to the 'Y' series of larvae (Y 1.2, Y 2.3, Y 3.4, instars) which do most of their feeding in the autumn, and which are nearly fully grown by the following spring. These larvae pupate in May and June to emerge as 'Y' beetles in the summer.

By the time the 'Y' beetles emerge, the plants are producing their second crop. After this has been picked the plants are normally ploughed up and larvae from eggs laid there by the 'Y' beetles eventually die, owing to lack of food. Many of the 'Y' beetles move to adjacent beds, however, which usually contain either newly planted runners, or plants from which the first crop has just been picked. The 'X' eggs which these beetles lay in the new site, hatch between July and September, giving rise to the 'X' series of larvae which, do most of their feeding in the spring, and pupate in the following late summer and autumn. They give rise to either 'X' beetles in the autumn or to 'X*' beetles which have pupated so late that it is too cold for them to emerge above ground, and which then remain in their pupal cells to emerge *en masse* in the spring. 'X' larvae do most of their feeding in the spring when heavy demands are being made on the roots of the plants for the formation of new leaves and fruit, so that the plants wilt just before fruiting. In general, new


June
May
July
Y P
·Y beetles
X 5 P
August
Y 5 P
April
Y eggs
Y beetles
X*beetles
X P
X beetles
X*beetles
X 3.4.5
Runners
X eggs
Y 1.2
Sept.
Mar.
Y 4.5
X*5 P
Feb.
X 1.2
Y 2.3
Oct.
X 2.3.4
X*beetles
Y 3.4
Nov.
Jan.
Dec.

Text-fig. 5. Diagram of life cycle of *O. clavipes* and strawberry plant hosts in Cheddar. X and Y—successive generations of weevils; X*—overwintering beetles; 1–5, larval instars; P, pupae.

runners are planted too late to be infested by any beetles before winter. The first infestation occurs in the following spring and summer and by the second year of plant growth both 'X' and 'Y' larvae are to be found beneath the plants.

The life history of the weevil occupies either 12 or 18 months according to whether it passes one winter as a fully grown larva, or one as a small larva and another as an adult beetle waiting to emerge from the soil. The longer life cycle is apparently a natural event. It is not brought about by disturbance of the beds when ploughed or cultivated, as it occurs equally in beds which are ploughed up after the second crop and in beds which are allowed to remain intact until the following spring.

CONTROL

In the Cheddar area the strawberry crop is the most profitable in the rotation and the one on which the economy of most holdings depends. The usual rotation is strawberries and anemones and other crops are only occasionally grown. The holdings are small, seldom more than 1 or 2 acres, and divided into strips which may even belong to different owners. In normal practice, the intensity of cropping is such that strawberries are grown in 2 years out of 3 in any particular strip and occupy two-thirds of the area in any field. The intensity of cropping and the difficulty of co-ordinating control measures even in a single field account for the increasing importance of *O. clavipes* in this area. The strawberries are apparently the only important host of the beetles at Cheddar and it is unlikely that either weeds or alternative crops harbour the beetles. The persistence of the infestations under the current system of rotation is fully accounted for by the emergence of beetles from old beds. Some of the more important *ad hoc* observations and experiments on control are described below.

Cultural control

Weevil attack can be avoided by growing strawberries in a 1-year rotation for some time. Runners planted in August usually escape infestation as the summer beetles do not have time to spread to the new beds before egg-laying finishes at the end of August. The plants remain clean during the following winter and are infested with beetles for the first time in the next spring. The larvae from eggs laid by these beetles are still quite small by the summer and cannot pupate without considerable further feeding. Thus, if the plants are ploughed up after the first crop has been picked, the infestation dies away. In spite of its drawbacks this method has been used with success to control the attacks on fields which are comparatively isolated from areas of heavy infestation.

As a general measure it is normally an advantage to leave damaged beds until the end of August or September if possible, so that the beetles emerging from them remain and oviposit *in situ*. Although the beetles themselves are not killed, their eggs are then laid in the old bed and the larvae are so small that they die of starvation when the plants are finally ploughed out.

Larvae in the soil are not killed by ploughing, and even when the plants are collected, normal methods of cultivation do not remove the whole of the roots. These roots provide food for the larvae, the largest of which pupate as usual in spite of the disturbance. A proportion of the remaining roots can be destroyed by repeated cultivation in hot dry weather but even then, many of the larvae pupate. Since normal cultivations do not kill larvae or pupae, attempts were made to squash them. Rows of damaged plants were turned out on to the hard ground between the rows with a single furrow plough and crushed with a Cambridge roller. This treatment was a comparative failure, since sampling showed that only half the older larvae were crushed, and large numbers of beetles subsequently emerged from this area.

The plants are always trimmed after fruiting and this is sometimes done by loosening the bedding straw and setting fire to it so that the plants and straw are burned together. The subsequent infestations have sometimes been much lighter on the burned portions than on the portions which were trimmed by hand. This method necessitates fine dry weather at the right time of the year so it cannot be used as a routine control measure.

The larvae are eaten by the larvae of the 'devil's coach-horse' (*Staphylinus olens*) and the beetles are often consumed in large numbers by badgers at night.

The likelihood of success of most cultural control methods is considerably reduced by the survival of older larvae under all but the most severe conditions and by the intensive cropping.

Chemical control

The adults

As beetles emerge more or less continuously throughout the summer, any treatment designed to control weevil damage solely through attacks on the adults must be repeated at frequent intervals. This is difficult, as certain insecticides cannot be used in May and June when the plants are cropping. The summer beetles are particularly difficult to kill economically. When the beetles are emerging from their pupal cells in succession, only a small proportion of the population are newly emerged and feeding on the plants at any time. The rest are egg-laying and remain in the soil for long periods so often do not pick up a lethal dose of insecticide before it is dispersed by the weather.

The spring beetles are more easily killed, as they all emerge and feed together for the first week or 10 days. As they collect in great numbers on the outside rows of plants at first, numbers can be significantly reduced by 10% DDT dust applied thickly to the ground beneath the first two or three rows of plants. The success of this operation depends on timing and it has been found most effective if the dressing is applied as soon as the first signs of leaf-eating appear and is renewed whenever necessary until the damaged leaves have been replaced by others which show no signs of damage.

Attempts were made to overcome the disadvantage of using dusts by the use of poison baits consisting of either raisins, apple pomace, or molasses and bran with sodium fluoride, sodium fluosilicate or lead arsenate as poison. In the laboratory some of the beetles were killed by these baits (Table 3) which were apparently eaten in preference to the plants on which the beetles were caged, but in the field the baits failed completely.

TABLE 3. *Number of* O. clavipes *adults out of forty (two replicates of twenty each) which were dead, moribund or alive after being caged for 48 hr. on strawberry plants around which baits had been distributed in the pots*

	Beetles		
Bait	Dead	Moribund	Alive
Molasses, bran, sodium fluosilicate	8	1	31
Apple pomace, bran, sodium fluosilicate	23	2	15
Raisins, bran, sodium fluosilicate	25	3	12
Raisins, bran, sodium fluoride	22	1	17
Raisins, bran, calcium arsenate	17	11	12
Bran, sodium fluosilicate	9	1	30
Control	0	0	40

The larvae

Larval mortality is difficult to estimate in the field as the crop is too valuable for extensive sampling. Consequently such investigations must be confined to small patches set aside for the purpose. On a field scale, in addition to skeletal sampling, careful observations are necessary to check both the yield and quality of the fruit and the numbers of beetles which subsequently emerge from the area, if the success of the treatment is to be assessed.

The larvae must be killed in time for the plant to replace sufficient of its feeding roots to give a satisfactory crop. As the feeding roots for a given crop are formed in the previous year, the larvae must be destroyed well before the winter. Fortunately they are then mostly young, and lie in the upper layers of soil where they are comparatively easy to reach with insecticides. Control measures have therefore been applied in late summer or early autumn.

In preliminary experiments 3rd instar or older larvae were used. Dusts were applied to the surface of the soil to be washed in by rain but gave no kill after several months. Systemic insecticides applied both to roots and leaves also failed. 10% DDT or lead arsenate was dusted into the holes in which the runners were planted: this checked the plants slightly by comparison with the controls, but no infestation subsequently developed on the plots.

In view of the success of such treatments against other strawberry weevils in Kent (B.D. Moreton *in litt.*) a trial was set up using liquid preparations of DDT, BHC and toxaphene poured on the soil at a pint per plant (see Table 4). The insecticides were applied through a rubber hose leading from a 40 gal. drum mounted on a small trolley which could be pulled between the rows. The treatments were carried out in the first week in September and some of them gave excellent results. Table 4 shows the results of sampling in the following February.

Treatment 2 has since been used on a field scale with complete success in some situations. The first field, which was treated in early September, consisted entirely of beds of strawberries of different ages, and although the older plants were heavily infested with grubs, a good crop was picked throughout, in the following year. When sampled in February after treatment, only one live larva was found in a sample of twenty plants taken at random from the 2-year-old beds, by comparison with the three or four larvae per plant found before treatment. Six beetles were found dead in their pupal cells, and no beetles emerged in the area, at least during the following spring and summer.

TABLE 4. *Total live larvae from under five plants taken at random from each replicate, in a randomized block of three replicates of five treatments for control of* O. clavipes *larvae* (*one row of eighty plants per replicate*). 1/1600 *additional wetter was added to each treatment*

	Grubs			
Treatment	Rep. 1	Rep. 2	Rep. 3	Total
(1) 1/100, 25 % miscible DDT	0	0	0	0
(2) 1/250, 15 % DDT emulsion with adjuvant	0	0	0	0
(3) 1/1000, Miscible BHC 10 % isomer	2	15	1	18
(4) 1/250, 15 % BHC emulsion with adjuvant	1	0	0	1
(5) 1/400, Toxaphene	13	10	6	29
Control	38	23	15	76

TABLE 5. *Number of live and dead larvae per row of forty plants after* 0·6 *c.c. DD had been injected into the crown of each plant in alterante rows*

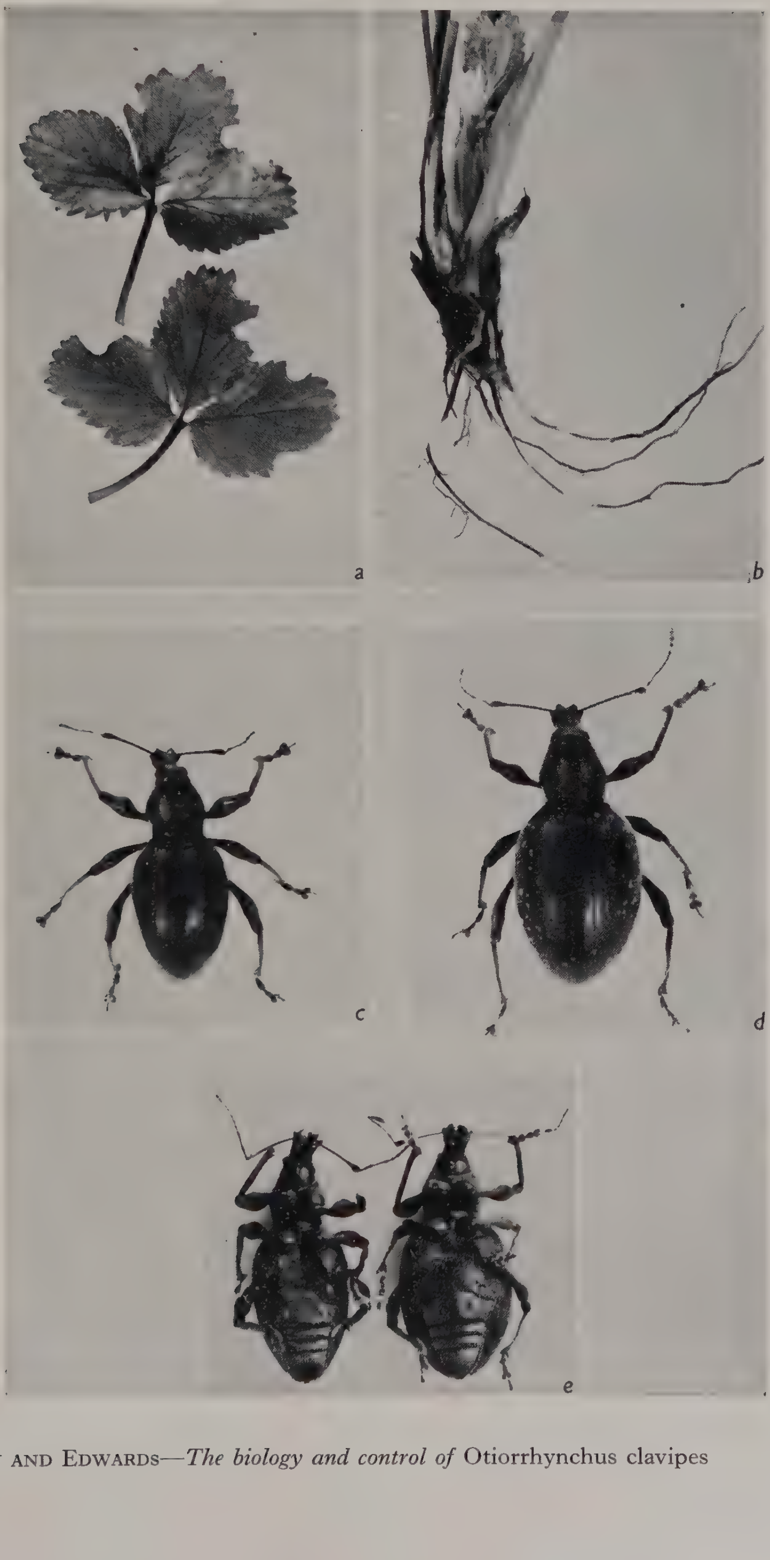
Row no. ...	1	2	3	4	5	6	7	8	9	10	11	12	13
	T	C	T	C	T	C	T	C	T	C	T	C	C
Larvae: Live	0	5	0	5	0	7	0	5	0	8	0	2	2
Dead	3	0	2	0	1	0	4	0	6	0	6	0	1

T—treated, C—Control.

In a further trial the grubs were also attacked by injecting the soil with DD (dichloropropane-dichloropropylene). In laboratory tests in a closed chamber fresh grubs were killed within 10 min. in air, and 6 hr. in soil, in the DD concentration which would be given around the roots of the plants, by an injection rate of 0·6 c.c. per plant in the field.

This injection rate was used in a field trial and killed both plants and grubs (Table 5). DD is a potential control for strawberry eelworm (L. N. Staniland *in litt.*) and it is possible that the treatment could serve a dual purpose in the future. At present it is a useful method of clearing small patches of infestation in an otherwise healthy bed.

Our thanks are due to Mr L. N. Staniland, the head of this department, who suggested the problem and who has greatly facilitated the work by his under-



Ibbotson and Edwards—*The biology and control of* Otiorrhynchus clavipes

standing encouragement; Mr J. E. Forshaw, County Horticultural Officer, Somerset, and Mr E. N. Powell his deputy for their helpful assistance; Dr A. M. Massee and Dr A. Hoffmann for information given in correspondence; and the growers who have all extended their fullest co-operation, in particular Mr J. Hanwell and Mr C. E. Hanwell of Draycott, Somerset, who placed strawberry beds at our disposal for experiments.

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EXPLANATION OF PLATE 16

O. clavipes adults and damaged strawberry plants: (*a*) leaves damaged by adults; (*b*) roots and stock damaged by larvae; (*c*) male adult; (*d*) female adult; (*e*) male (L.) and female (R.) showing curvature of posterior thoracic sterna.

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REVIEWS

General Virology. By S. E. Luria. Pp. 427+xiv, with 93 figures. New York: John Wiley and Sons Inc.; London: Chapman and Hall Ltd. 68*s*.

Except for its title, which suggests the biography of a successful although little-known soldier, most things about this book can be welcomed enthusiastically. It is the first attempt to present the study of viruses as a basic science that is no less suitable for teaching to students than, say, mycology or bacteriology, and the attempt undeniably succeeds. Its success derives primarily from Professor Luria's unusually wide knowledge of viruses that infect different kinds of organisms and from his firm conviction that those that attack animals, bacteria and higher plants have more in common than an ability to cause disease, but an important contributory reason is that he has set himself strictly defined limits. He does not concern himself with clinical details, and emphasizes similarities between the behaviour of different viruses rather than differences. His prime purpose is to relate the results of physico-chemical studies on viruses *in vitro* with what is known about the physiological and bio-chemical changes they induce while they multiply in infected cells. Sometimes this entails assuming that what has been discovered with one virus will also apply to another; in this way the subject gains a pleasing air of uniformity that may justify itself by simplifying teaching, but also has its dangers. Perhaps the greatest danger is that students may consider knowledge to be much greater than it actually is, which is certainly not the author's intention; rather by stressing similarities, he intends to suggest that lines of work that have proved profitable with a virus affecting one kind of host are worth following with other viruses.

It would not be difficult for the specialist research worker to criticize some sections of the book adversely, but to do so would be to mistake its main function, namely to outline for students the varied techniques used in the study of viruses and to summarize current views on the nature of viruses and their method of multiplication. There are errors, but some errors are almost inseparable from generalizations and from the condensing necessary to fit the subject into a book of reasonable size. Workers on plant and animal viruses may also consider that the book is unbalanced and gives a disproportionate amount of space to bacterial viruses. But these are Professor Luria's speciality, they provide him with his model for viruses that attack other organisms, and they are also the most convenient subjects for class exercises. Certainly though, if students can assimilate the detail and argument in the chapter entitled 'Genetic analysis of phage reproduction', they might reasonably be expected to take more than two pages on the transmission of plant viruses by insects and more than the one page under the heading 'Viruses and plant metabolism'. However, perhaps it is as well this last section is no larger, for most of the few statements it now contains are misleading. Here, as elsewhere, some inaccuracies might have been avoided by stating what has been observed, instead of giving interpretations as though they were facts.

Professor Luria's stated intention was to produce a class book, but he has done more than this and there are few who have been engaged in virus research who will not benefit from reading it. Techniques are described simply and clearly, and those who do not need his excellent advice on pipetting, the use of logarithms or the analysis of assays, will probably find something they did not know in his accounts of the chick embryo, of tissue culture, the haemagglutination phenomenon, or of lysogenic bacteria. Workers on animal, bacterial and plant viruses have occasionally met to their mutual advantage at conferences, but usually they continue as though they were engaged in separate disciplines. Perhaps the greatest achievement of this book is to show that there is one instead of three subjects and to provide those who work in one branch with a painless method of getting information about the other two.

The last chapter deals with the *Rickettsia* and seems oddly out of place, particularly as the author states that the similarity between them and viruses 'does not go very far'. The book is well produced, clearly printed on good paper, and the many excellent illustrations are chosen judiciously. There is a bibliography of more than 700 references, and a detailed subject index.

F. C. BAWDEN

Control of Rats and Mice. Edited by DENNIS CHITTY and H. N. SOUTHERN. Vol. I, *Rats*: Pp. xxxii + 306 with 4 plates and 18 illustrations in text. Vol. II, *Rats*: Pp. xvi + 307–532 with 4 plates and 11 figures in text. Vol. III, *House Mice*: Pp. xvi + 226 with 8 plates and 21 figures in text. Oxford: Clarendon Press; London: Oxford University Press. 1954. 105*s*. the set.

Modern methods of rodent control owe much to the wartime researches of Charles Elton and his colleagues at the Bureau of Animal Population at Oxford. Therefore, although some of the results of their investigations have already been published the appearance of the present volumes is an event long awaited by other workers in this field.

Owing to the unfortunate delay in publication, the circumstances of which are explained in the preface, some paragraphs are already out of date. This, however, is of small moment since the main purpose at any rate of the two volumes on rats is not to provide an easy guide to control, but to illustrate the difficulties inherent in this type of research, to show how certain problems were solved and how others, still unsolved, can be approached.

Vols. I and II are edited by Chitty and are indexed separately from vol. III. Volume I begins with an introduction by Elton which is followed by a chapter by R. B. Freeman and others on the properties of the poisons used in rodent control. This section of the book contains much useful information, but of course has nothing to say about the new anti-coagulant poisons which are to-day widely used here and in other countries.

The second half of vol. I consists of a long chapter by Chitty on the study of the brown rat, *Rattus norvegicus*, and its control by poison. This is by far the most valuable chapter in the whole work and should be read over and over again by all engaged or interested in research on rat control. After a discussion of existing census methods, the food and water requirements of *R. norvegicus* and the factors influencing bait-take, the author describes the technique of, and justification for, the now classic method of census by surplus baiting. Other subjects treated at some length are methods for comparing the efficiency of various poisons, the advantage of prebaiting before poisoning and the phenomenon of poison and bait shyness in rats which have been fed sublethal doses.

Whatever future advances may be made in rat-control, the methods of study described in chapter 4 will remain a model for a long time to come. It is unfortunate therefore that the last two pages of vol. I show an unwillingness to recognize the merits of the anti-coagulant poisons mentioned above. Their success in certain types of environment is so patent that to carry out a long series of comparative trials against the older rodenticides would be (to borrow Chitty's own words) 'to seek intellectual satisfaction at the expense of practical objectives'.

The second of the two rat volumes contains chapters by eleven workers and includes such topics as the reactions of *R. norvegicus* to environmental changes (Monica Shorten), rural rat control (A. D. Middleton), the results of sample censuses of rats and mice in corn-ricks over four years (C. Elton and E. M. O. Laurie), the control of rats in sewers (T. S. Perry), and the control of *R. rattus* in London (Perry and J. S. Watson). On some of these subjects work could not be carried far: nevertheless, every chapter contains valuable information.

The third volume is devoted to the house mouse, *Mus musculus*, and is edited by Southern. This also is the result of collaboration between a number of workers. Unlike the volumes on the rat, vol. III does set out to provide a guide to control and even gives a key to procedure in dealing with different types of mouse infestation. This is not, however, the main purpose of the book which is rather to describe the work done on the home range and other aspects of the ecology of *M. musculus*, its status as a pest, its feeding activity and food preference and its reactions to various methods of control, particularly poisoning. The restricted home range of the house mouse and its less marked response to prebaiting techniques compared with the rat are well brought out and illustrate the difficulties still to be overcome in this field.

All three volumes are well documented and contain, for example, more than 150 tables. Five guineas is a lot of money even for three books but, set against the cost of the damage caused annually by rats and mice in Britain, it is of little consequence. It is to be hoped therefore that every Medical Officer of Health in the country will read these volumes and then hand them on to his Sanitary Inspectors.

E. W. BENTLEY

An Introduction to Industrial Mycology, 4th edn. By G. SMITH. Pp. 378. London: Edward Arnold (Publishers) Ltd. 1954. 30*s*.

The ubiquity of the common mould fungi, in all climates and on all kinds of organic material, make this group of interest to all who are concerned with storage problems. In addition, their useful activities are being harnessed to give an ever increasing number of industrial fermentations.

Many of the organisms belong to the Fungi Imperfecti, a group regarded as vaguely indecent by the older text-books on mycology, and passed over as briefly as possible. Nevertheless, the majority of 'spoilage' moulds come into this group, and the industrial worker welcomes a book that gives it due attention.

Mr Smith's book has become a standard work of reference on the commoner mould fungi. The present edition has been largely rewritten, and shows an increase of more than 100 pages over the 1946 edition: there are also a number of new photographs, including some of additional genera.

As in previous editions, one of the principal attractions is the unrivalled series of photomicrographs. Admittedly these have their dangers, and one encounters certain people who identify their mould isolations by 'comparing with the pictures in Smith'. It is only fair to say that the text gives ample warnings against such facile optimism, and offers references to a wider literature.

The revised introductory chapter shows the influence of Langeron's recent views. Whether fungi should or should not be classed with the Plant 'Kingdom' has been a debating point since the days of Linnaeus, and nowadays leaves most mycologists unmoved.

Sections dealing with morphology, classification and nomenclature have been thoroughly revised. Two features for which beginners in mycology will be grateful are (1) a clear summary on the rules of nomenclature, and (2) etymological notes on the meaning of names and technical terms. A strong plea is put up for a sensible pronunciation of names that does not obscure their meaning. One remembers the efforts of misguided purists to stress 'penicillin' on the second syllable instead of the third; such efforts were ultimately defeated by the development of other antibiotics—polymyxin, gramicidin and others—in which the principle became increasingly difficult.

Chapters IV to VII dealing with Zygomycetes, Ascomycetes and Fungi Imperfecti have all been extended and improved. Recent Dutch work on the classification of the yeasts is admirably summarized and illustrated; but *Pityrosporium* on p. 73 should read *Pityrosporum*. A useful key to the commoner genera of moulds is given at the beginning of chapter VIII, and the individual genera are then more fully described in alphabetical order. *Aspergillus* and *Penicillium* require more detailed treatment and each occupies a separate chapter. That on *Penicillium* includes a key to the common species, and additional aids to identification.

Of the more general sections, that on Laboratory Equipment and Technique has been most expanded. Of techniques, like watches,

> '.........none
> Go just alike, yet each believes his own',

and this applies especially to the technique of making subcultures. But the long experience behind chapter XI will be appreciated by all practical microbiologists.

Later chapters deal with mould physiology, culture collections, control of mould growth, and industrial uses of fungi. Millardet, who was ever at pains to establish his priority in introducing copper sprays for vine mildew, would have been displeased to have seen this attributed to Pasteur (p. 312). The final chapter gives an annotated list of mycological works of reference, and a new appendix has been added on microscopy and photomicrography.

This workmanlike revision of an extremely useful book will be widely welcomed.

L. D. GALLOWAY

Methods of Surveying and Measuring Vegetation. By DOROTHY BROWN. Pp. 233. Bulletin 42, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire. 1954. 15*s*.

Qualitative and quantitative assessments of vegetation entail the patient resolution of many varied factors and conditions, and the methods which have been devised to study the problems involved are, of necessity, very numerous and often highly individual. No single worker in this field will have had first-hand experience of but a few of the techniques available. Indeed, many of these techniques being widely scattered in the literature will have escaped attention, and the critical comparison and evaluation of methods for the study of new and problematic areas, as well as for the development and improvement of established methods has been difficult. There has therefore been much need for a single presentation of the subject, and ecologists, agronomists and advisory service staffs, will find the present work an invaluable clearing house of the information and experience now available in this field.

Miss Brown's work is confined mainly to grassland vegetation in the widest sense, and covers minutely all aspects of recording, including botanical analysis, productivity (both herbage and animal production methods) as well as the assessment of management and utilization practices. Each method is described in detail, and as far as the literature allows, its uses and limitations are discussed. No single research worker or reviewer could hope to cover this aspect adequately, but the evaluations presented here will serve as a valuable preliminary guide to investigators in appreciating the cardinal features of the various techniques. There is also an informative chapter contributed by Mr G. M. Jolly on the theory of sampling.

The book is pleasant to read and, with a few exceptions, notably in chapter 3, the writing is unambiguous. The author can feel very satisfied in having achieved a remarkable unity of composition from the diverse and somewhat unresponsive material at her disposal. Many readers will, however, find the definitions given for 'plot' and 'line' on pages 19 and 20, to be different from the accepted sense and general usage of these terms and, therefore to be somewhat confusing. It is to be earnestly hoped that an immediate and general revulsion will be felt against the use of the irritating term 'forb' which has been borrowed from the U.S.A. to denote a herb possessing useful forage qualities.

The volume is illustrated by numerous tables, plates and figures; there is a glossary of terms and an extensive bibliography.

WATKIN WILLIAMS

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EXPERIMENTS ON BLACK LEG DISEASE OF SUGAR-BEET SEEDLINGS

By L. F. GATES and R. HULL

Rothamsted Experimental Station (Dunholme Field Station, near Lincoln)

(With 9 Text-figures)

Phoma betae was usually the predominant pathogen attacking sugar-beet seedlings in the field in eastern England, but *Pythium* spp. sometimes predominated when the seedlings were very young. *Fusarium* spp. were associated with acid soil conditions. *Rhizoctonia solani* only occasionally caused severe losses.

Black leg, caused chiefly by *Phoma betae*, was most prevalent at low soil temperatures when, in stands from untreated seed, up to 45 % of the seedlings were attacked. Disease decreased by 0·8 to 2·4 diseased seedlings per 100 in stands from untreated seed per 1° F. rise in soil temperature. Seed treated with Panogen,* thiram and ethyl mercury phosphate regularly gave better stands in small-scale trials than seed treated with Ceresan or Agrosan.

In large-scale experiments average increase in seedling emergence following treatment with Panogen, thiram and Agrosan varied in different years between +6·4 and +19·2 %. Average response to BHC soil dressing was +4·2 and +8·6 %. Response to seed dressings increased as the time taken for emergence increased and as percentage emergence decreased; when emergence was 50 % of the possible, dressing gave on average 20 % more seedlings. Response to dressing increased as soil became more acid or alkaline than the optimum for beet, and was lower on heavy soils and on soils dressed with farmyard manure.

Maximum emergence varied from year to year between 2100 and 2700 seedlings per oz. seed; minimum between 690 and 1050. Emergence of both treated and untreated seed varied greatly and was related to time taken for emergence, suggesting that effects of soil moisture, temperature or texture are important.

---

* *Footnote key:*

Agrosan GN	Mixture of phenyl mercuriacetate with ethyl mercurichloride (total Hg = 1 %)
Aldrin	1:2:3:4:11:11 - Hexachloro - 1:4:5:8:9:10 - hexahydro - 1:4:5:8 - diendo-methylene-naphthalene
BHC	Benzene hexachloride (γ-isomer)
Ceresan UT 1875*a*	Methoxyethyl mercurisilicate
Ceresan (U.S.A.)	2 % ethyl mercury chloride
Ceresan M	7·7 % ethyl mercury *p*-toluene sulphonanilide
Cuprocide	Cuprous oxide
Dow 9B	Zinc trichlorophenate
Gammexane	Benzene hexachloride (98 % γ-isomer)
Granosan M	7·7 % ethyl mercury *p*-toluene sulphonanilide
New improved Ceresan	5 % ethyl mercury phosphate
Panogen	Methyl mercury dicyandiamide
Phygon	2:3-Dichloro-1:4-naphthoquinone
Spergon	98 % tetrachloroparabenzoquinone
Thiram	Tetramethylthiuram disulphide

Emergence was not correlated with pH, but when the pH was below 6·5 or above 8·7 it was not more than 75 % of that at pH 7·0–7·4. In 2 out of 3 years emergence was increased by 32 and 84 seedlings/oz. seed by each extra cwt. per acre of inorganic fertilizer (N, P, K). Seed rate, cultural operations and drill type had no consistent effect.

A 1 % increase in seedling stand increased the final plant population on average by 0·2 %.

Black leg, caused by seed-borne or soil-inhabiting fungi, kills sugar-beet seedlings between the time the seed germinates and the time the hypocotyl begins secondary thickening. All seed sown for the British sugar-beet crop is now given a fungicidal dressing and black leg is rarely responsible for extensive losses; in recent years British Sugar Corporation fieldmen have reported only 200 to 700 acres out of 400,000 destroyed or severely damaged. The losses in most crops are unimportant because the seed rate usually gives 5–20 times the number of seedlings required to produce a full stand after singling. Death of unhealthy seedlings after singling may be of more consequence. However, black leg might become more important if practices that reduce the initial number of seedlings are generally adopted (Harvey, 1952).

The fungi most frequently attacking beet seedlings are seed-borne *Phoma betae* and soil-inhabiting fungi, mainly species of *Pythium*, *Aphanomyces*, *Fusarium* and *Rhizoctonia solani*. The relative importance of these pathogens varies in different beet-growing areas, and, consequently, the conditions favouring disease and the benefits from seed treatment also vary. *Phoma betae* is of minor importance in the United States and Canada, where, in central and eastern regions, *Aphanomyces cochlioides* is the most important pathogen, being favoured by high soil temperature and abundant moisture (McKeen, 1949). *A. cochlioides* is little affected by seed dressing but its effects are mitigated by abundant fertilizer, especially phosphate, by suitable crop rotation and by soil treatment with thiram (Afanasiev, 1948*a*, *b*; Coons, Kotila & Bochstahler, 1946; Hildebrand, McKeen & Koch, 1949; Hildebrand & McKeen, 1950). In Western U.S.A. *Pythium*, *Rhizoctonia* and *Fusarium* spp. are more important, and both dry and wet seed treatment with Ceresan, New improved Ceresan, Ceresan M, ethyl mercury phosphate, Cuprocide, Phygon, or formulations of thiram, can increase emergence but may not maintain a good stand (Gaskill & Kreutzer, 1946; Leach, 1948, 1949). In north-west Europe seed is harvested in damper conditions favouring the seed-borne *Phoma betae*.

Phoma attacks more severely when seedlings grow slowly (Edson, 1915; Petherbridge & Stirrup, 1935). Leach (1947) showed that low temperature is less unfavourable to *P. betae* than to sugar-beet seedlings. Jacks (1951) found that more diseased seedlings occurred at 5–12° C. than at 16–20° C., and more when the soil moisture was increased from 25 to 40 and 65 % of water-holding capacity. Among the best materials for use as seed dressings against *P. betae* are various mercurial dressings, especially organo-mercury compounds, with formulations of thiram equally as good or somewhat better and Spergon and cuprous oxide less efficient

(Woodward & Weston, 1929; Hughes, 1935; Grente, 1950*a*, *b*; Jacks, 1951; Jorritsma, 1951). McKay (1948) states that in Eire dressing sugar-beet seed before distributing it to farmers appears to have doubtful value in checking losses from *P. betae*. Soaking the seed in ethyl mercury phosphate, however, has given good results (Cadogan, 1952).

I. RELATIVE IMPORTANCE OF THE PATHOGENS

In each year from 1936 to 1939 in the east Midlands most beet fields examined contained some seedlings with black leg, the percentage attacked ranging from less than 1 to more than 40%. Diseased seedlings were surface sterilized by dipping in 0·1 % mercuric chloride for $\frac{1}{2}$–$\frac{3}{4}$ min., washed in sterile water and plated out on agar, made with half tap water and half distilled water acidified with malic acid to pH 6·5. By this means, a number of pathogenic fungi were isolated. Although *Pythium* appears fairly sensitive to mercuric chloride its rapid growth on tap-water agar enables it to outgrow other fungi. Several species of *Pythium* were isolated but generally it was *P. intermedium* de Bary or *P. ultimum* Trow.

In isolations made in 1937 and 1938 from diseased seedlings in Lincolnshire, *Phoma betae* was the most prevalent fungus (Table 1). From one typical batch of seedlings affected with black leg, the following fungi grew from the lesions: *Phoma* alone from 33, *Pythium* and *Phoma* from 7, *Pythium* alone from 3 and no fungi from 2 seedlings. Similar results were obtained from two later examinations of the same field.

TABLE 1. *Isolations from diseased beet seedlings, 1937 and 1938*

		Percentage of plants giving							
Disease	No. of fields	*Phoma*	*Pythium*	*Phoma* and *Pythium*	*R. solani*	*Fusarium* sp.	*Alternaria* sp.	Not identified	No fungus
Black leg	17	48·0	16·1	4·2	3·7	0·2	0·0	4·2	23·5
Acid soil injury	12	25·1	9·2	0·6	1·6	30·8	2·9	20·3	9·5

Only occasionally was *Pythium* predominant, and this generally when the seedlings had only just emerged. In a field of this type, the following fungi were isolated: *Pythium* alone from 19, *Phoma* alone from 4, no fungus from 4 seedlings. By contrast, when diseased seedlings were taken from the same field a fortnight later, *Phoma* was predominant, and *Pythium* was obtained from only a very few seedlings. Probably most of the young seedlings attacked by *Pythium* had died.

In an experiment made in a Lincolnshire loam soil in 1939, the relative abundance of *Phoma* and *Pythium* was observed at various times (Table 2). In the early-sown plants *Pythium* predominated at the first sampling and *Phoma* at the second. The early sowings also had more plants infected with *Pythium* than the later ones; this has been found in other experiments on Lincolnshire loam soils. In the two later sowings of the 1939 experiment *Phoma* predominated throughout, except when Ceresan seed-dressing reduced *Phoma* more than *Pythium*.

Fusarium spp., mainly *F. angustum* and *F. culmorum*, (Table 1) were the predominant pathogens on seedlings with stringy hypocotyl, one of the symptoms of acid soil injury (Hull, 1950). This trouble, which may occur on soils up to pH 6·5, annually causes failure or more than 25 % reduction of stand on 1600–2800 acres. It is primarily induced by the soil acidity, and the *Fusarium* spp. are only weakly parasitic. In pot experiments, beet growing in acid soil inoculated with cultures of the two fungi developed stringy hypocotyl only after 2 months. The two fungi were recovered from the affected hypocotyls. In other experiments with a species of *Fusarium*, the fungus could not be induced to attack healthy seedlings.

TABLE 2. *The relative importance of* Phoma *and* Pythium *on beet seedlings in an experiment in* 1939

Seedlings per 100 infected with	Sown 15 April				Sown 2 May				Sown 27 May			
	Sampled 5 May		Sampled 18 May		Sampled 20 May		Sampled 2 June		Sampled 15 June		Sampled 1 July	
	U*	T†	U	T	U	T	U	T	U	T	U	T
Black leg	9·8	2·2	20·7	3·2	20·5	5·5	3·2	2·5	6·0	3·7	5·8	1·5
Phoma‡	1·7	0·0	12·9	1·6	11·0	1·3	1·5	0·5	1·5	0·8	3·1	0·2
Pythium‡	5·3	0·8	0·9	0·0	3·5	2·1	0·5	0·8	0·3	0·0	0·0	0·2

* U = untreated seed.
† T = Ceresan treated seed.
‡ Remaining seedlings with black leg gave neither fungus.

The superficial mycelium of *Rhizoctonia solani* was killed by dipping in mercuric chloride, so that seedlings had to be examined for this fungus before they were surface sterilized. It was detected in seven of the twenty-nine samples in Table 1, but it was the predominant fungus in only one. *R. solani* was found in 1951 in nineteen samples from sites showing poor stands, in six of which it was apparently the main cause of injury. In 1952 only seven samples showed *Rhizoctonia*, and in three it appeared to be the main cause of injury. In 1953 seven samples showed *Rhizoctonia*, including two samples from areas in Norfolk, where it occurred on many acres of unthrifty beet on light land but may not have been the primary cause of poor growth. In most seasons *R. solani* seldom causes severe losses. It occurs on both light and heavy soils at all pH's from 5 to 9, but samples were more frequently received from acid or alkaline soils than from neutral ones.

II. THE EFFECT OF TEMPERATURE AND MOISTURE ON THE SEVERITY OF SEEDLING DISEASE

In 1938, at Sutton Bonington, three strips each of thirty rows were sown, fifteen with untreated seed and fifteen with seed treated with an organo-mercury compound. The soil was heavy loam overlying river gravel. The strips were sown on 19 April and 3 May. The seedling stand was counted and examined for black leg. In 1939 plots arranged in a 6 × 6 Latin square on the same type of soil were sown with untreated or Ceresan-treated seed on 15 April, 2 and 27 May. Black leg was estimated just after emergence and 10 days before singling. In 1949 an experiment at Dunholme, Lincs, on limestone loam included four sowing dates, 1 and 13 April, 2 and 24 May, and two rates of phosphate dressing (0, 8 cwt./acre) on eight plots in each of four blocks. On each plot one row was allotted to each of the twelve combinations of seed lightly infected with *Phoma* and seed heavily infected; untreated seed and seed dressed with Panogen or Ceresan, and soil untreated or treated with thiram. The rows contained 150 seeds of which 100 were for counts of the seedlings emerging and fifty were for estimation of the proportion diseased. An experiment in 1950, on limestone loam, consisted of three blocks, each of six plots; the plots being used for six different sowing dates (28 March; 6, 19, 28 April; 11, 17 May). On each plot were randomized twelve rows of untreated seed and twelve of Panogen-treated seed, which were used one at a time for sampling at 2 to 3-day intervals, starting immediately after emergence.

Soil temperature was measured at a depth of 4 in. in 1938 and 1939 and 1 in. in 1950. In 1949 daily maximum and minimum air temperatures were measured. The proportion of seedlings with black leg was determined by examining washed seedlings from sample lengths. This method does not measure pre-emergence losses or seedlings which are killed and disappear. Conditions favouring rapid death of infected seedlings might leave fewer infected seedlings to be counted at sampling than conditions favouring only a less severe attack. Seedling numbers could give full losses, except that the different soil conditions at different times of sowing may affect germination as well as disease incidence. Increased number of seedlings resulting from seed dressing only partially measures the number of seedlings killed because some loss also occurs with treated seed. However, as large losses of seedlings in experimental plots have always been correlated with a high proportion of black leg in the survivors, estimations of percentage diseased seedlings when the first true leaves are just beginning to expand are a reasonable estimate of disease severity.

In 1938 (Fig. 1) black leg was present from the time when the first seedlings emerged until the middle of May. During this period there was little rain, winds were mainly cold and soil temperatures were low. Later in May when rain fell and the soil temperature rose to between 50 and 60° F. black leg was not found. The seedling life of the first sowing coincided with black leg occurrence and significantly more plants were obtained from treated than from untreated seed. The second sowing was made during the cold period, but germination was delayed by dry soil and the seedlings grew in warmer weather; seed treatment slightly increased seedling population. Seedlings of the third sowing also grew in warm, moist conditions and the untreated seed gave more seedlings than the treated seed. In the 1939 experiment (Fig. 2) most disease occurred when the soil temperature was between

45 and 55° F., and less when it was between 55 and 65° F. Rain fell during both the colder and the warmer periods. In all sowings seed treatment reduced black leg and in the first increased the stand from 15·8 to 20·8 seedlings per yard. In the second, treated seed gave fewer seedlings per yard (18·1 against 23·1) and in the third sowing there was no difference (treated 26·2, untreated 26·5).

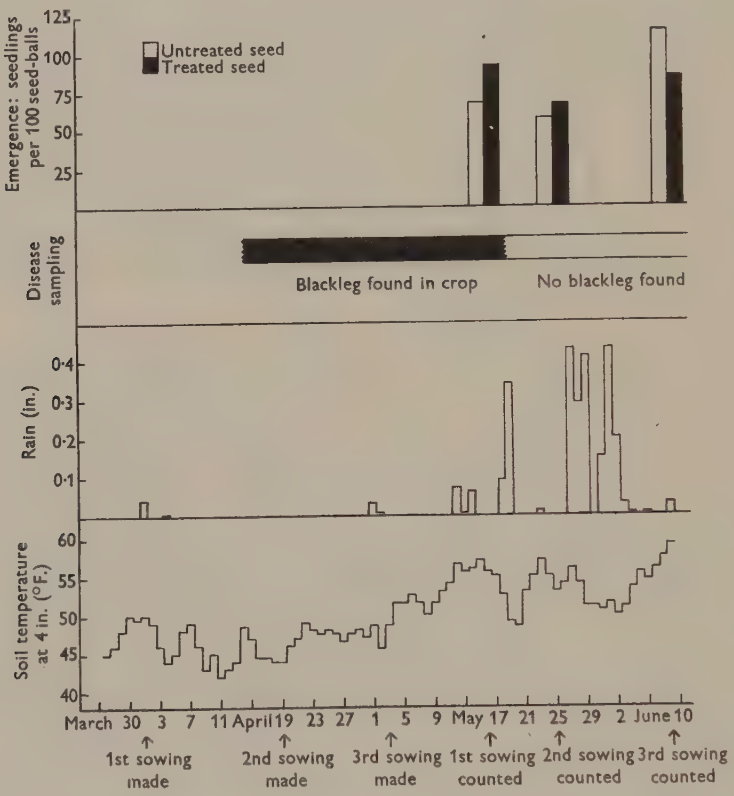

Fig. 1. Black leg and weather in 1938.

The first sowing in 1949 (Fig. 3) germinated during a spell of good weather in mid-April and had less black leg than the second sowing. The disease decreased from the second to the fourth sowings as the temperature rose. Seed dressing reduced the percentage of infected seedlings by about half in all the sowings. The first and third sowings responded most to seed dressing, the second showing a smaller response in seedling number despite the higher disease percentage (Table 3). The fourth sowing showed least increase in seedling numbers. In 1950 (Fig. 4) soil temperatures were low and rose only slowly during April and May, and the soil moisture remained between 11 and 19% of soil weight. Not until the beginning of

TABLE 3. *Effect of seed dressings in an experiment in* 1949. *Beet seedlings per* 100 *seed-balls*

	Sowing dates				
	1 April	13 April	2 May	24 May	Mean
	±2·24*		±2·59†		
Untreated	70·4	63·3	64·4	77·4	68·9
Ceresan	83·8	71·6	81·8	86·1	80·8±1·12
Panogen	88·8	74·6	82·7	86·9	83·2
Mean	81·0	69·9	76·3±1·83	83·5	77·7

* S.E. for vertical comparisons.
† S.E. for comparisons other than vertical.

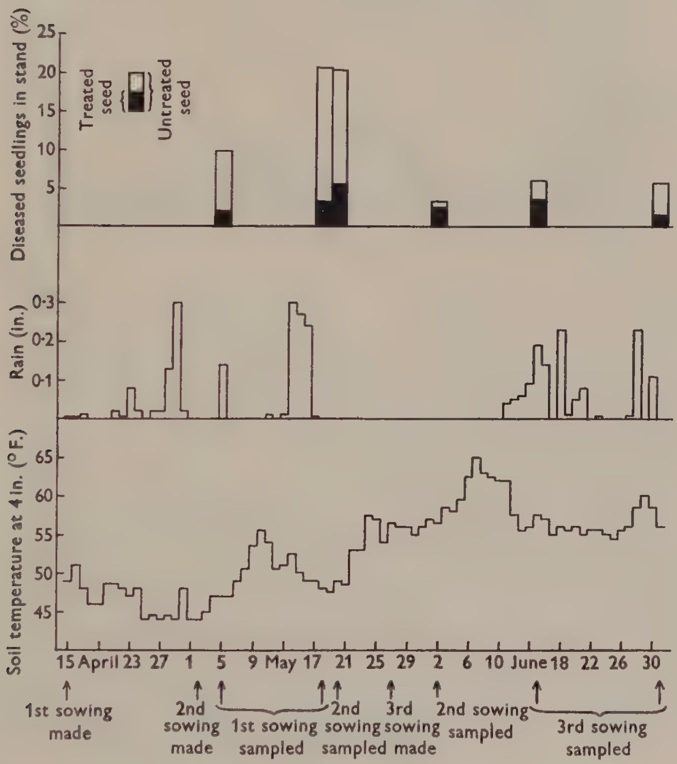

Fig. 2. Black leg and weather in 1939.

June did the soil temperature rise and the soil moisture fall markedly. Throughout the seedling life of the sixth sowing, in which the disease was much less than in the previous sowings, the soil temperature at 1 in. varied from 60 to 80° F. and the soil moisture between 5 and 10% of soil weight. The proportion of plants with black leg from untreated seed fell progressively from the first to the third sowings, then increased, to become less again in the fifth and much less in the sixth (Fig. 4). On the Panogen plots the trend was similar except that the fourth and fifth sowings had fewer infected plants than did the first three. Panogen reduced the disease level to between a fifth and a seventh of that of the untreated stand at all sowing dates.

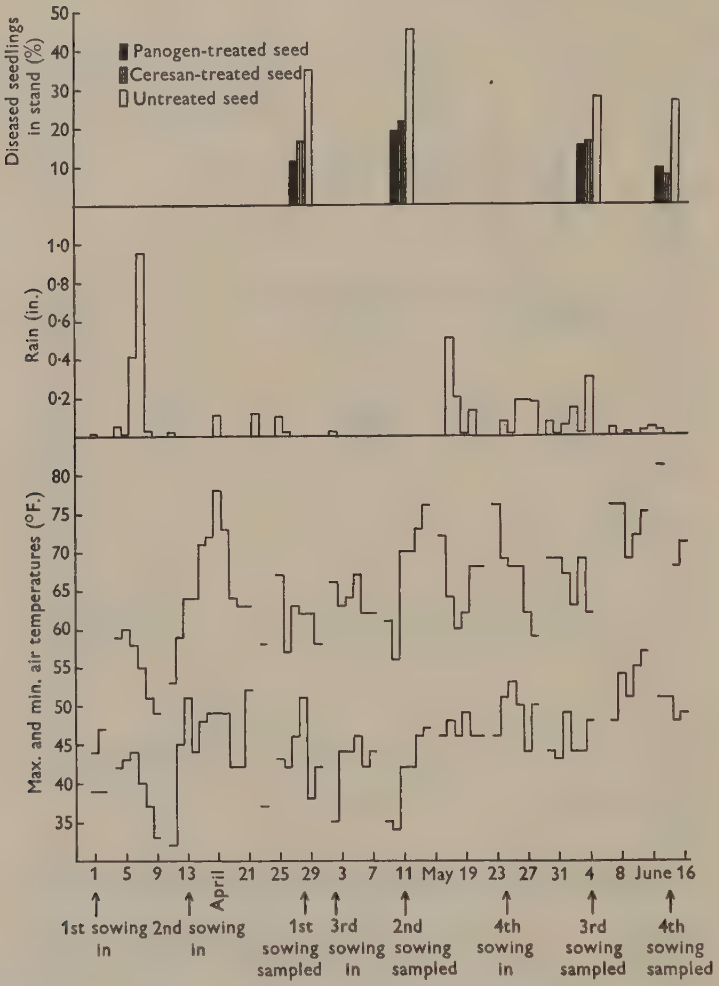

Fig. 3. Black leg and weather in 1949.

The relations between disease incidence and temperature and moisture are summarized in Table 4. Increase in soil temperature consistently reduced the proportion of diseased seedlings in the stand. Maximum disease encountered in stands from untreated seed was 21–45 diseased seedlings per 100. Disease decreased by 0·8 to 2·4 diseased seedlings per 100, per 1° F. rise in soil temperature. For treated seed black leg varied from 5 to 23 diseased seedlings per 100. Disease decreased (except 1939 Ceresan) by 0·3–1·5 diseased seedlings per 100 per 1° F. rise in temperature. The effects of increased soil moisture varied according to the relation between soil temperature and moisture. Increased soil moisture was accompanied by increased disease in 1939 and 1950 but by less disease in 1949 and 1938.

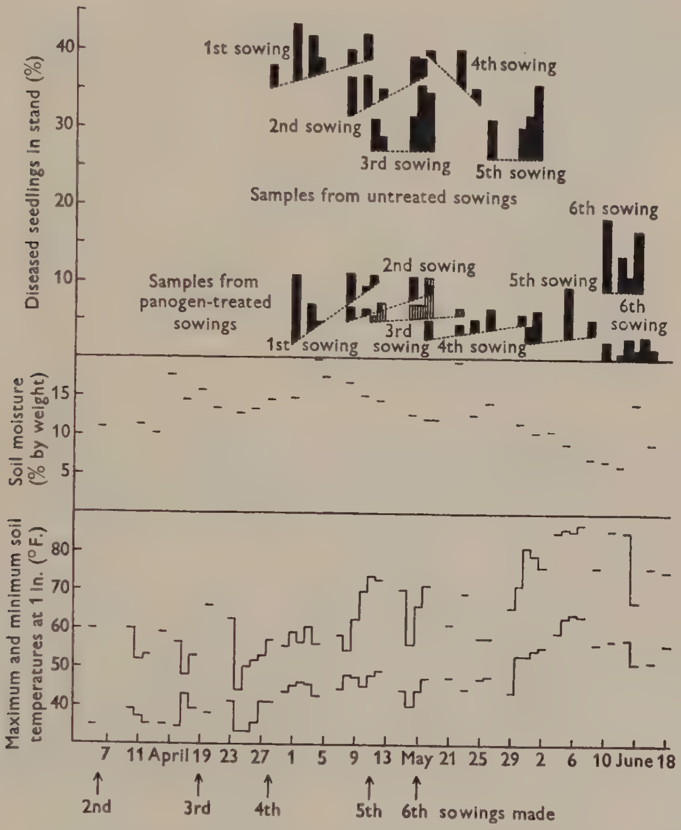

Fig. 4. Black leg and weather in 1950. The histograms show the disease at its highest level in each sowing.

Seed dressing seems to decrease disease incidence by a similar factor in all sowings of any one experiment. Thus in 1939 untreated sowings had from 3 to 5 times as many diseased seedlings as treated sowings, in 1949 about twice and in 1950 about 6 times as many as treated sowings. Seed dressing gives a better stand response when the seedlings are under adverse conditions because a larger proportion of them are then attacked.

TABLE 4. *Black leg and weather conditions* 1939, 1949 *and* 1950

Year	Seed dressing	Range of disease encountered (% seedlings diseased)	Range of temperature encountered (° F.)†	Range of moisture encountered†	Change in % diseased seedlings per degree rise in temperature	Change in % diseased seedlings per increase of 0·01 in. in daily rain or per 1 % increase in soil moisture	Change in temperature per increase of 0·01 in. in daily rain or per 1 % increase in soil moisture
1939	None	20·7– 3·2	45·7–59·7, soil temperatures at 4 in.	0·0–0·069 in. rain daily	−0·8**	+2·4**	−0·90
1949	None	45·0–28·0	62·2–70·8 (max.), 41·8–49·3 (min.), air temperatures	0·0017–0·074 in. rain daily	−2·1** −2·4**	−2·4**	+0·90 +0·86
1950	None	41·0–14·8	56·1–82·1 (max.), 38·8–58·5 (min.), soil temperatures at 1 in.	8·5–15·8 soil moisture, %	−0·93** −1·3	+3·1**	−0·26**
1939	Ceresan	5·5– 1·5	45·7–59·7, soil temperatures at 4 in.	0·0–0·069 in. rain daily	−0·01	+0·11	See above for 1939
1949	Ceresan	22·8–10·6	62·2–70·8 (max.), 41·8–49·3 (min.), air temperatures	0·0017–0·074 in. rain daily	−1·4** −1·5**	−1·1	See above for 1949
1949	Panogen	20·0–11·0	62·2–70·8 (max.), 41·8–49·3 (min.), air temperatures	0·0017–0·074 in. rain daily	−1·0* −1·1*	−0·7	See above for 1949
1950	Panogen	8·9– 1·7	56·1–82·0 (max.), 39·0–58·3 (min.), soil temperatures at 1 in.	8·6–15·7 soil moisture, %	−0·27** −0·38**	+0·84**	See above for 1950

*, **, denote regression coefficients significant at $P=0·05$ and 0·01 respectively.
† Over 2 weeks previous to sampling date (3 weeks in 1950).

III. RESPONSE TO SEED TREATMENT

Small-scale seed dressing trials were made from 1949 to 1952 at Dunholme, Lincs. The seed had a fairly high *Phoma* infection. The trials were made (with one exception) in a limestone loam soil, in which *Pythium* has been seldom encountered on beet seedlings except in early sowings. Neither *Rhizoctonia solani* nor acid soil injury were observed. Seed (30–340 g.) with fungicide doses varying round that recommended by the makers was shaken by hand for 5 min. in flasks or bottles of about twice the seed volume. Randomized block designs were used, one row being allotted to each treatment. The best method found for sowing was to place 5–7 g. rubbed seed in a Planet drill, which was set to empty itself in approximately 40 ft.; a further 5 ft. of row allowed loose seeds to be cleared by hand from the drill. By this method the plot S.E. for seedling counts was ±9% in 1951 and ±13% in 1952. Large responses in seedling number to seed dressing were accompanied by a relatively high proportion of diseased seedlings in the controls.

The increases in seedling number for Ceresan at 1½ or 2 oz./bushel or Agrosan at 0·67% of seed weight were 23% of control (untreated) in 1949, 9% in 1950, 34 and 25% in 1951, 33·4% in 1952 and 53% in 1953. In each year methyl mercury compounds gave the best results. Corresponding percentage increases for Panogen (66 c.c./kg.) in 1949–52 were 31, 13, 80 and 64, and 47. A dose of 12 c.c./kg. was less effective and 18 c.c./kg. deleterious in 1950. Methyl mercury nitrate, at 2 oz. per bushel, also gave good results but is too dangerous for commercial use. Only one of several other experimental mercurial dusts tested gave results better (in 2 years out of 3) than the routine mercurial dressings. Formulations of phenyl mercury dinaphthyl methane disulphonate gave results equal to or nearly equal to routine mercurial dressings. Phygon was tested for 5 years, with doses ranging from ¼ to 3% of seed weight, but gave only moderate increases in stand, not as good as Ceresan or Agrosan in 1949, 1950 and 1952, but better in 1951 and 1953 when used at 1% (+52%, +40% and +76%). Thiram at 0·33% active material on natural seed gave results in 1950 and 1951 close to those of Panogen, but in 1952 and 1953 on rubbed seed was little different from Agrosan. Granosan M (0·25%) and Dow 9B (0·5%) equalled Agrosan in 1952 but were better in 1953 (+60%, +74% respectively). Soaking seed for 20 min. in a 40 p.p.m. solution of ethyl mercury phosphate gave very good results (+45% in 1952 and +70%, +75% in 1953). In two other trials in 1953 ethyl mercury phosphate gave average increases of 120 and 49% compared with 87 and 35% for Agrosan.

IV. EXPERIMENTS ON THE CAUSE AND CONTROL OF VARIATIONS IN SEEDLING EMERGENCE IN THE FIELD

Experiments have been made to determine: (1) the effect of seed dressings on emergence and final plant population in commercial crops; and (2) the relative importance of seedling disease, insect injury, soil conditions and cultivation in determining plant stand.

In 1936 and 1937 experiments were done on five soil types. Two coulters of a four-row drill were supplied with Ceresan-treated seed and two with untreated seed. The machine therefore planted four-row strips alternately with treated and untreated seed. Seedlings were counted which occurred within three 4 in. lengths marked on a 30 in. stick placed at random in the row. The benefit from seed dressing sometimes equalled that obtained in small-scale trials, but in both years the variation in emergence from site to site was considerable despite the seed dressing (Tables 5 and 6).

TABLE 5. *Beet seedling emergence and response to seed dressing in 1936*

No. of days after sowing	Seedlings per 100 seed-balls sown					
	Wold loam. Sown 20 April		Gravelly loam. Sown 8 April		Light sand. Sown 15 April	
	Control	Ceresan-treated	Control	Ceresan-treated	Control	Ceresan-treated
22	98	111	—	—	—	—
26	—	—	78	78	72	78
29	88	105	—	—	—	—
33	—	—	77	79	—	—
41	—	—	73	77	—	—

TABLE 6. *Beet seedling emergence and response to seed dressing: effect of seed dressing on final plant population in 1937*

Type of Soil ...	Sandy loam		Acid sand		Sandy loam		Sandy loam	
Origin of Klein E seed ...	Continental		Continental		English		English	
	Control	Ceresan-treated						
	A	B	A	B	A	B	A	B
Seedlings per 100 seed balls (just before singling)	102	120	92	121	46	73	51	67*
Percentage of seedlings with black leg (shortly after emergence)	34	17	38	8	—	—	—	—
Seedlings per yard (just before singling)	21·4	27·7	27·7	33·8	25·2	25·7†	24·9	34·3*
Difference	6·3 ± 2·71		6·1 ± 2·23		0·5		9·4 ± 2·01	
Plants per chain (after singling)	57·4	63·1	60·3	61·4	59·5	58·4†	61·4	68·8
Difference	5·7 ± 1·13		1·1		−1·1		7·4 ± 1·64	
Final plant population (thousands per acre)	25·3	27·8	23·9	24·3	23·6	23·1†	22·9	25·6
Difference	+2500		+400		−500		+2700	

* Counted just after germination.

† Fifty-five seed clusters per yard were sown in the control plots, compared with thirty-five in the treated plots.

In 1949, 1950 and 1952 further experiments were carried out by the fieldmen of the British Sugar Corporation at the following places:

1949

Great Welnetham, Suffolk
Spixworth, Norfolk
Kilconquhar, Fife
Pymoor, Ely, Cambs
Saffron Walden, Essex
Tunstall, Suffolk
Astley, Worcs
Fakenham, Norfolk
Thorney, Northants
Bilton, Yorks
Hatfield, Yorks
New Milton, Hants

1950

Stanton, Shropshire
Crowle, Lincs
Spixworth, Norfolk
Dura Mains, Cupar, Fife
Pymoor, Ely, Cambs
Harlow, Essex
Dunham on Trent, Notts
Much Dewchurch, Hereford
Tatterford, Norfolk
Thorney, Northants
Wetherby, Yorks
Basingstoke, Hants

1952

Stanton, Shropshire
Ulceby, Lincs
Brandon, Suffolk
Elsing, Norfolk
Newburgh, Fife
Pymoor, Ely, Cambs
Finchingfield, Essex
Trent Lane, Notts
Leominster, Herefordshire
Tatterford, Norfolk
Yaxley, Northants
Wetherby, Yorks
Wistow, Yorks
Broughton, Hants

In each year a site was chosen representative of the predominant soil type of the area. The seed was Sharpe's Klein E fairly heavily infected with *Phoma*. In 1949 and 1950 one-third was treated by machine with Agrosan, another third with Panogen and the rest left untreated. Seed rate and cultivations were those normally used by the farmer. To give a measure of the activity of soil pests, 2 cwt./acre Plant Protection wireworm dust (containing Gammexane) were applied either on the day of sowing or a few days before or after.

The experiment consisted of six strips each eight rows wide, extending across the field. Each seed treatment was sown on two strips, the three treatments being arranged in two blocks. There were two areas treated with Gammexane dust, each $54\frac{1}{2}$ ft. wide extending across a block of 3 seed-treatment strips. Thus in each experiment there were twelve sampling plots for plant counts. Those not on the Gammexane areas were chosen at least 10 yards away from them to avoid Gammexane drift.

On each of the twelve plots four sets of counts were made: (1) as soon as all seedlings had emerged; (2) just before the braird was gapped; (3) immediately after singling; (4) when the crop was finally established. For counts 1 and 2 the number of seedlings was counted on three 1 ft. lengths of each of the eight rows of the strip within the sampling plots. The counts were evenly distributed along the length of row used for sampling, but the actual 1 ft. lengths were selected at random. For counts 3 and 4 the procedure was similar, but the number of plants in three 11 ft. lengths of each of the eight rows was counted.

In 1952 rubbed seed was used at a seed rate usually of 7–9 lb./acre. Seed dressings were aldrin, thiram and aldrin, and Agrosan, Panogen and thiram used alone and in combination with BHC. Each treatment was duplicated and the experiment consisted of twenty strips each four rows wide extending right across the field. Counts were made on two occasions only, when seedling emergence was complete and when the final stand was well established.

Agrosan in 1949 gave increased stands at ten and Panogen at eight of the twelve sites. The increases varied up to 50% of the control (Fig. 5). The average response to Agrosan over the twelve sites was +13·5%, that for Panogen was +19·2%. In 1950 responses to seed treatment were smaller, Agrosan increased stands at eight of the twelve sites and Panogen at five out of twelve sites. Over the twelve sites the average response to Agrosan was +6·4% and to Panogen −5·2%. This result with Panogen differs from previous experience, and its failure may reflect uneven application on the seed, causing damage to some that was overdressed (cf. seed-dressing trial results). In 1952 the average response to Agrosan was +7·2% when used alone and +15·2% when combined with BHC. Corresponding figures for Panogen were +7·9 and +7·7%, and for thiram were +7·3 and +19·2%. Agrosan and thiram alone improved the stand at nine and eight respectively of the thirteen centres; and with BHC at twelve and thirteen centres. Panogen gave improved stands at ten of the thirteen centres both with and without BHC. The average response to thiram was little greater than to Agrosan.

Responses to dressings are usually small (Fig. 5) compared with differences due to other factors, which are discussed later; treated seed varies almost as much in emergence as does untreated seed. Response to seed dressing varies, however, from site to site, although seed from the same batch was used for all the experiments of each year. Fig. 6 shows that when conditions did not favour germination and growth, responses to seed treatment were greater, and that when as few as 1000–1300 seedlings emerged from an oz. of seed, the dressings gave an average of 20% increase. Fig. 6 includes the 1952 results because an experiment in 1950 showed that a sample of seed gave approximately the same number of seedlings per oz. after rubbing as before rubbing; the lower number of seeds per cluster being compensated by the increased number of clusters per ounce. Response to seed dressing in each year also increased as the time taken for emergence of the seedlings increased: 1949, +1% per day ($P = 0·1–0·2$); 1950, +0·6% per day (not sig.); 1952, +1·7% per day ($P = 0·1$). Emergence and time taken for emergence of seedlings were themselves correlated in 1949 and 1952. Fig. 7 shows that better responses to seed dressing occurred on soils with a pH somewhat unfavourable for beet growing, particularly on acid soils. Emergence and pH were not themselves correlated in these experiments. The figure also shows that, of the acid soils, light sands or peats

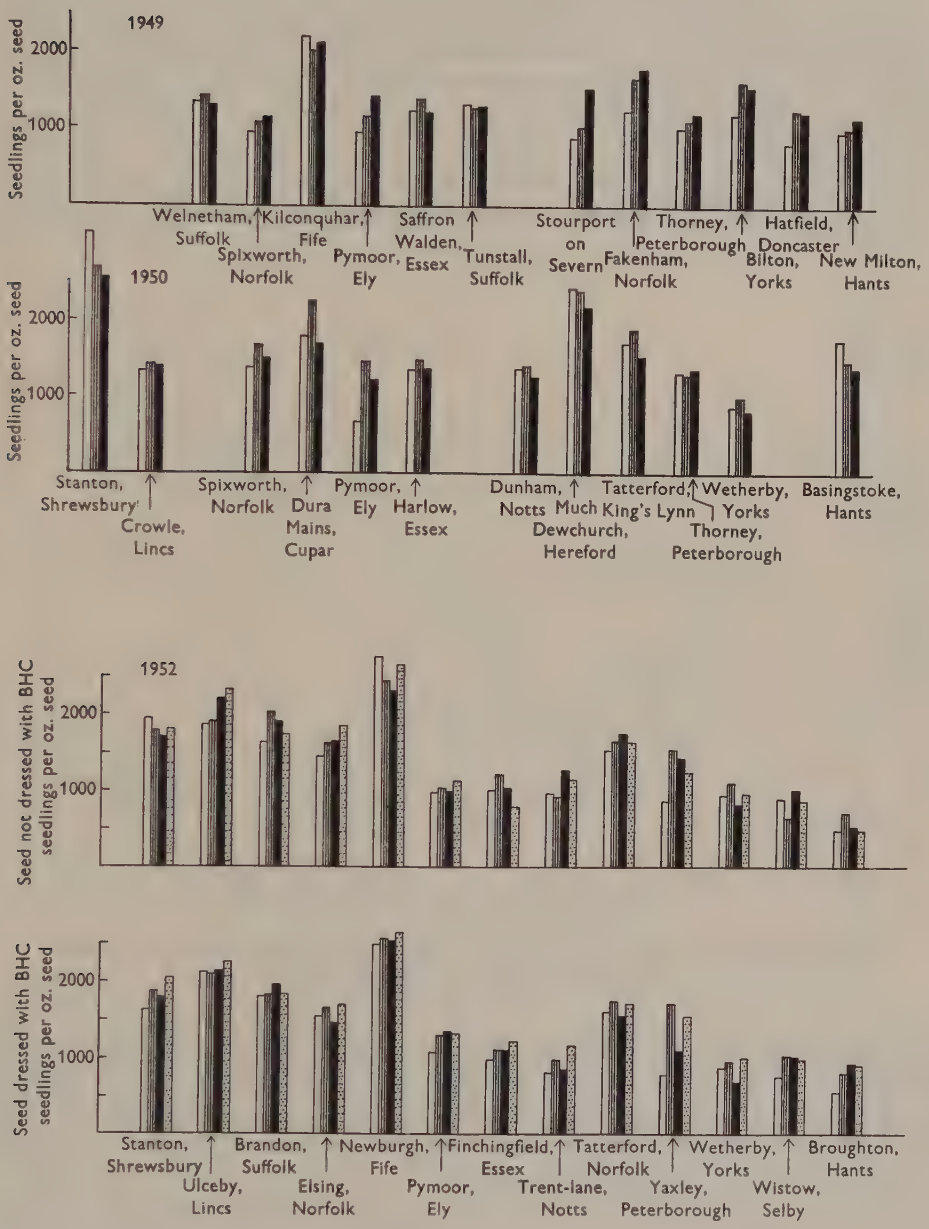

Fig. 5. Increases obtained from seed dressings in commercial beet crops. White columns = untreated seed; shaded columns = Agrosan-treated seed; black columns = Panogen-treated seed; stippled columns = T.M.T.D. (thiram)-treated seed.

(circles) tend to give responses above average, whereas heavier soils (squares) give responses below average. In acid soils, particularly the lighter ones, soil-inhabiting fungi may be more active in killing seedlings (Garrett, 1944, pp. 44–7), and the soil acidity may render seedlings more susceptible to fungi. Similarly, at pH 8·8–9·0, beet seedlings sometimes have poor colour and grow slowly, possibly because the high pH renders nutrients unavailable. Eight of the thirty-two sites giving above-average response to seed dressing received farmyard manure compared with twenty-four out of the forty-six showing below-average response (Fig. 7). Inorganic nitrogen, phosphate and potash fertilizers were applied at all sites: in 1949 and 1950 they had no effect on response to seed dressing, but in 1952 seed-dressing response decreased with increased fertilizer (– 1·2 % per 1 cwt./acre fertilizer (P=0·1–0·2)). However, fields where small quantities of fertilizer were applied were probably sufficiently fertile for seedling requirements.

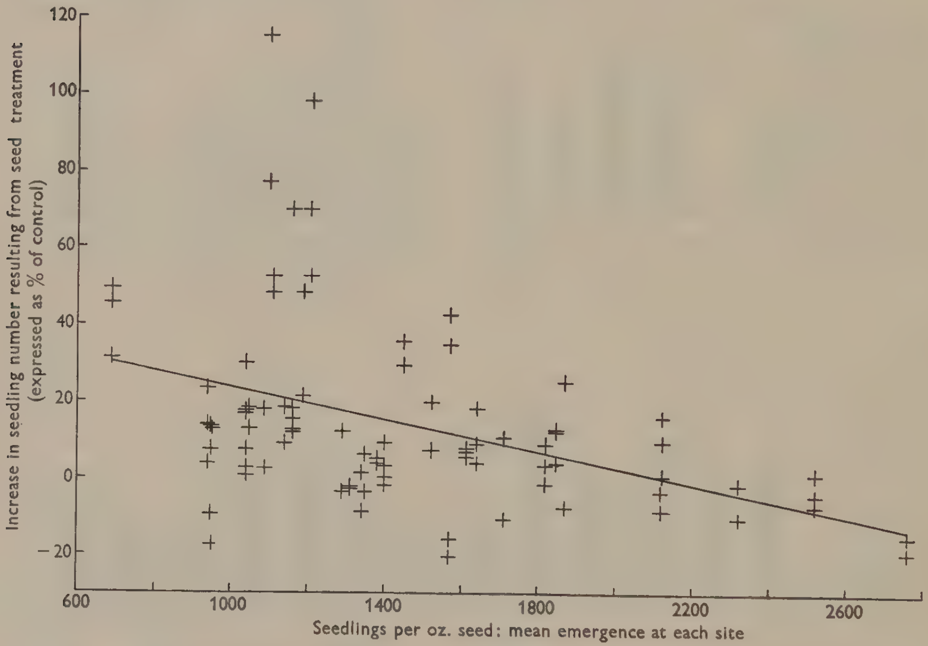

Fig. 6. Relation between response to beet seed dressing and seedling emergence (regression sig. at P=0·001).

In 1949 the mean response to the Gammexane soil dressings for the twelve sites was +4·2, compared with +13·5 and +19·2 for the fungicidal seed dressings (Fig. 8). In 1950 the mean response was +8·6 % compared with +6·4 % for Agrosan. In these experiments the responses to treatment against soil pests are of the same order as those to seed dressings, but the fields selected, although not picked for low wireworm populations, were likely to be less infested than average, whereas the seed was selected because it was infected with *Phoma*.

Only at a few sites was the response to seed and soil treatment sufficient to indicate that diseases or pests played a major part in determining the number of seedlings emerging (Figs. 5, 8). Emergence varied greatly whether seed was treated or not. At most sites each year responses to seed treatment were within ±20% of the controls and to Gammexane dust within ±10%. The seedlings produced from an ounce of seed (averaged over all treatments) varied from 2120 to 1050 in 1949, 2760 to 950 in 1950 and 2564 to 691 in 1952. Similar variation in emergence is reported by Gahan (1953), Jones (1953), and Jorritsma (1953), who consider that soil physical conditions are largely responsible. In our experiments emergence was smaller the longer the seedlings took to emerge; 1949 (excl. Cupar), −12 seedlings per day's delay in emergence ($P=0{\cdot}1$); 1950, −50 seedlings per day's delay ($P=0{\cdot}01$); 1952, −135 seedlings per day's delay ($P=0{\cdot}01$). This suggests that checks to emergence depend largely on conditions that hinder seedling growth, i.e. adverse

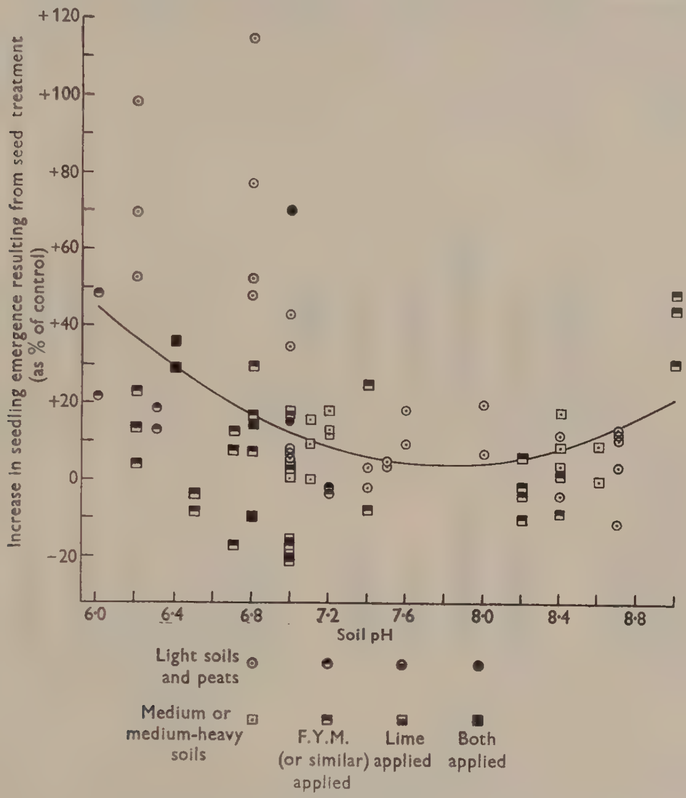

Fig. 7. Response to beet seed dressing at various soil pH's (overall regression sig. at $P=0{\cdot}01$).

soil texture, temperature and moisture conditions. No consistent effect of seed rate, cultural operations or drill type has been traced. Average emergence on light soils was 1400, on the heavy soils 1550. The best stands of seedlings were obtained at pH 7·0–7·4, while below 6·5 and above 8·7 the stands in these experiments did not exceed 75 % of those at 7·0–7·4. However, within the range 6·5–8·7 and even at 7·0–7·4 there were many cases where germination was only 50 % of the optimum. Emergence also showed some correlation with the amount of inorganic fertilizers (N, P, K) applied; in 1949, +32 seedlings for each additional cwt. per acre ($P=0\cdot05-0\cdot1$); 1950, +18 seedlings per 1 cwt./acre (not sig.); 1952, +84 seedlings per 1 cwt./acre ($P=0\cdot01-0\cdot02$).

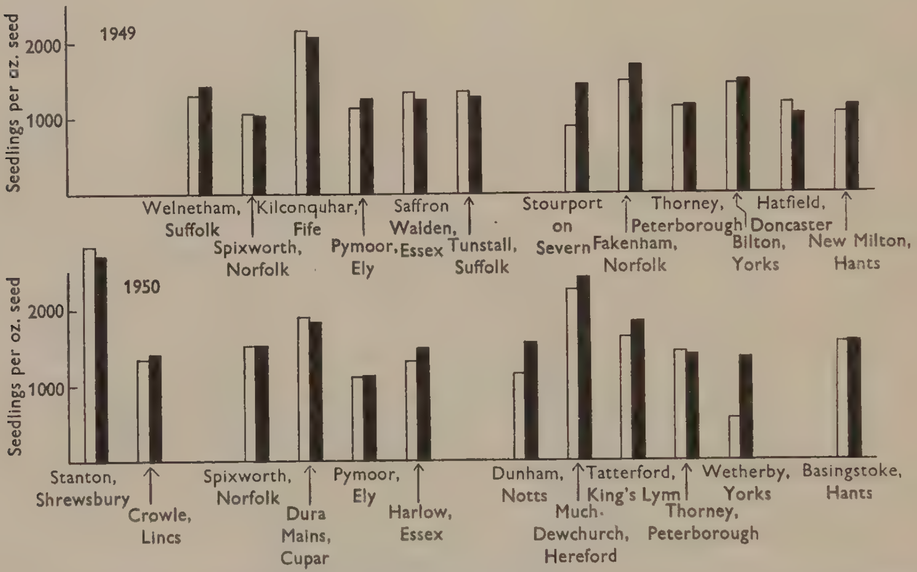

Fig. 8. Increases obtained from soil dressings of BHC in commercial beet crops. White columns = soil not treated; black columns = soil treated with Gammexane dust.

There was no evidence of post-emergence losses in these trials in 1949. In the 1950 trials post-emergence losses ranged up to 24 %, the mean loss being 9·8 % (Table 7). Seed treatment and Gammexane dust have only slightly reduced these losses; they are probably largely due to cultivations. Losses after singling were usually small.

Variations in seedling stand had a significant effect on final plant population. In Fig. 9 increases in seedlings per yard at the first count on the 'Agrosan' and 'Panogen' plots (due to slight differences in seed rate as well as seed dressings) are expressed as percentages of the 'untreated' stand and compared with the corresponding increases in plants per chain (fourth count) expressed in like manner. Some figures from 1936 to 1939 are included. The points on the graph show

TABLE 7. *Post-emergence losses of beet seedlings in 1950*

	Percentage increase (+) or decrease (−) of number of seedlings between first and second counts*					
	Whole experiment	Plots without Gammexane dust	Plots with Gammexane dust	Plots without seed treatment	Agrosan-treated seed	Panogen-treated seed
Stanton, Shrewsbury	−22·8	−26·2	−19·3	−22·8	−24·6	−21·2
Crowle, Lincs	−8·7	−6·4	−11·0	−8·5	−9·9	−7·8
Spixworth, Norfolk	−24·1	−12·9	−35·3	−22·2	−26·4	−24·1
Dura Mains, Cupar	+5·2	+3·2	+7·3	+9·0	+0·68	+6·2
Pymoor, Ely	+5·5	+8·5	+2·5	+4·3	+3·8	+7·7
Harlow, Essex	−23·1	−26·4	−20·2	−25·1	−22·6	−21·7
Dunham on Trent, Notts	−1·7	−3·6	−0·4	−7·3	+3·6	−1·7
Much Dewchurch, Hereford	−12·3	−13·5	−11·3	−13·0	−10·5	−13·0
Tatterford, King's Lynn	−9·6	−10·9	−8·5	−15·4	−9·1	−2·4
Thorney, Peterborough	−6·0	−13·3	+1·2	−5·2	−3·4	−8·8
Wetherby, Yorks	+7·8	0·0	+11·1	+5·2	+3·0	+15·5
Basingstoke, Hants	−19·6	−22·1	−17·8	−24·6	−18·7	−18·3
Mean	−9·8	−11·3	−8·5	−11·0	−10·3	−8·2

* First count: as soon as seedling emergence was completed. Second count: just before the braird was gapped.

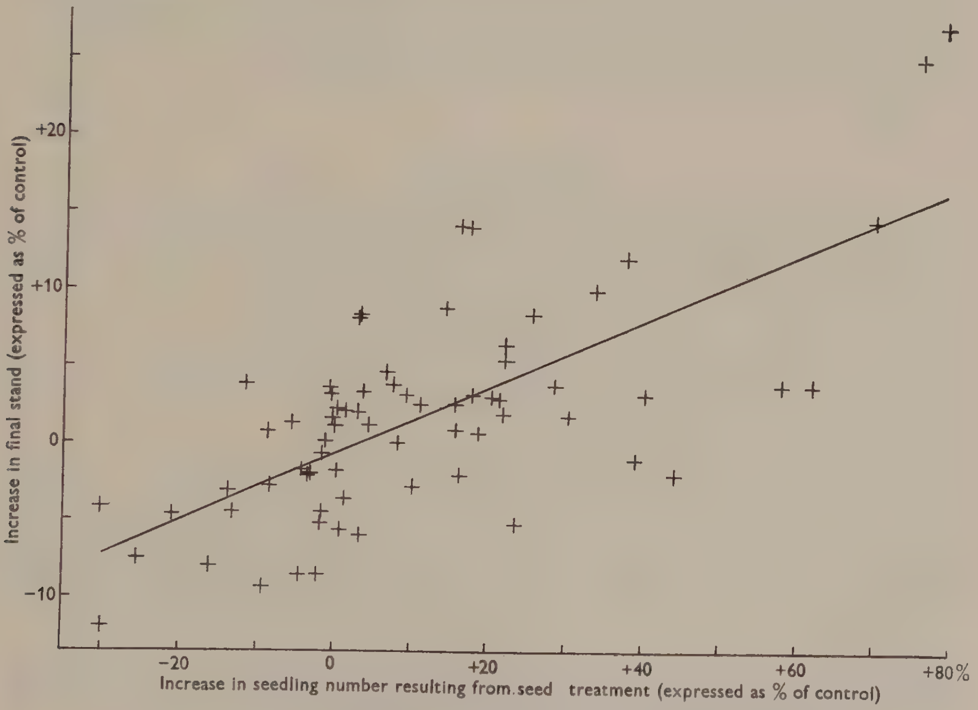

Fig. 9. Effect on final stand of increases in beet seedling population (regression sig. at $P = 0{\cdot}001$).

considerable scatter because the number of seedlings present in the braird for each plant finally required varied from 26·2 down to 4·9 in 1949 and 28·2 to 5·5 in 1950, and also because the final population aimed at by the hoemen varied from 25,600 to 36,700 plants/acre in 1949 and from 21,800 to 38,700 in 1950. Regularity of the braird and skill of the hoemen in compensating for irregular seedling stands are important. The populations actually achieved varied in 1949 from 67 to 99% and in 1950 from 70 to 107% of those aimed at.

The significant linear regression of increase in final stand on increase in seedling population resulting from seed dressing (Fig. 9) shows that, in these experiments, for each 1% increase in seedling stand the final stand of plants was increased on the average by 0·2%. With precision drills and very low seed rates the final stand is likely to be more sensitive than this to irregularities in the braird. The average increase in seedling numbers due to the seed treatments in these experiments varied from +6·4 to +19·2 which would increase the final stand by 1·4 to 4·1%. In cases where seedlings are increased by 30–50%, the increase in final stand would be 6–10%, showing the present 'insurance value' of seed dressing.

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THE USE OF SEED DRESSINGS CONTAINING γ-BHC IN THE ESTABLISHMENT OF SUGAR-BEET SEEDLINGS

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(With 2 Text-figures)

From 1949 to 1952 field trials were made to determine the effect of seed dressings containing γ-BHC, with and without the addition of organic mercurial compounds, upon the establishment of sugar-beet seedlings. Most of the trials were made in fields where no serious pest problems were anticipated. The 1949 trials established the concentration of γ-BHC non-phytocidal to sugar-beet seedlings. Later trials tested the effect on pre- and post-singling plant populations of dressings applied to natural or rubbed seed sown at standardized rates. Parallel tests of dressed seed were made on farms representing a variety of soil types. It was found that the phytotoxicity of γ-BHC varied with season and soil type, but that a dressing containing 40% was usually safe.

In these trials, the benefits from seed dressing were, as a rule, slight. Generally the combined mercury-γ-BHC seed dressing gave the best pre-singling stand of plants. Most of the improvement was attributable to the organic mercury rather than to the γ-BHC, but where pest attacks occurred, γ-BHC gave marked improvements in stand. Wireworm appeared to be the chief pest controlled: control of pygmy mangold beetle was only partial.

Extensive trials carried out by Jameson, Thomas & Woodward (1947), Arnason & Fox (1948), Jameson, Thomas & Tanner (1951), Jameson & Callan (1951), and Price Jones (*in litt.*) have shown that seed dressings containing γ-benzene hexachloride (γ-BHC) protect cereal and sugar-beet crops from the attacks of wireworms (*Agriotes* spp.) after ploughed up grass. In 1949–52, the authors tested the value of γ-BHC seed dressings in the establishment of sugar-beet seedlings on arable land.

Wireworms wound the hypocotyl below ground at a point sufficiently near the seed cluster (at normal depths of drilling) to be exposed to a volatile insecticide previously applied to the cluster. The pygmy mangold beetle (*Atomaria linearis* Steph.) and the spotted snake milepede (*Blanjulus guttulatus* Bosc.) also attack the hypocotyl below ground and might be similarly controlled. Wireworm attack after grass may be anticipated, but sporadic attacks also occur in light arable land, especially in East Anglia, where damage may be aggravated by adverse growing conditions. A higher seed rate (15–20 lb./acre) may compensate for the depredations of pests, but current practice favours lower seed rates, processed seeds, precision drills and the mechanization of singling. The use of γ-BHC seed dressings against seedling pests acting at low intensity had not previously been tested and so was made the subject of the investigations reported below.

Fungicidal seed dressings are well established and the basic problem is to incorporate into them a percentage of γ-BHC sufficient to control seedling pests without causing phytotoxicity. For sugar beet with its low seed rate (15 lb./acre), a higher concentration of insecticide and a higher dressing rate are required than for cereals where the seed rate is higher (150 lb./acre). This higher loading of insecticide on the seed increases the risks of phytotoxicity.

METHODS

The seed dressings used in these experiments included technical grade γ-BHC in a kaolin base plus a small quantity of non-phytotoxic oil to suppress dustiness and improve adherence. Organic mercury compounds were also incorporated. Except for four of the demonstration strips in 1949, for which Bush E seed was used, the variety Sharpes British Klein E was employed throughout. The dressings were applied with a churn-type machine, 7 g./kg. seed being the normal dressing rate. Standardized plant counts were made on seedlings at the 2-leaf stage, immediately before singling and on establishment, and are referred to as counts 1, 2 and 3, respectively, in the tables of results.

THE 1949 TRIALS

In 1949 three full-scale field trials, one small-scale trial and six demonstration strips were laid out. They were designed mainly to test the phytocidal rather than the insecticidal effects of γ-BHC and were, therefore, with one exception (trial 2) on fields where no serious pest infestation was anticipated.

Field trials

In the full-scale trials, seed dressed at 3·5 or 7 g./kg. with a 70% γ-BHC seed dressing, with and without the addition of an organic mercurial compound (Hg) as in the proprietary product Agrosan GN,* was compared with undressed seed and seed dressed with mercury only. In the field the seed was sown with a hand-drill at two rates, normal (L) and twice normal (H). This gave in effect twelve soil treatments, since the higher seed rate doubled the deposit of γ-BHC and Hg on certain plots. The seed and soil treatments are listed in Table 1. The twelve soil treatments were randomized independently and replicated 4 times, giving 48 plots with a total area of half an acre. Table 2 gives details of the individual sites and Table 3 shows the results of plant counts.

The best pre-singling stands were most often obtained from combined dressings containing γ-BHC and Hg but the increases over the controls receiving no dressing (0) were largely due to the Hg and only to a small extent to the γ-BHC. A mildly phytotoxic effect is suggested by the reduced stands obtained with γ-BHC in trial 3. In trial 2, where the wide spacing gave the heaviest deposit of γ-BHC per yard of row,

* Mixture of phenyl mercuriacetate with ethyl mercurichloride (total Hg = 1%).

plants here and there showed symptoms of toxicity on the high seed rate plots receiving two doses of γ-BHC (9 mg./yd. row).

TABLE 1. *The seed and soil treatments applied in trials 1–3, 1949*

Seed treatments				Soil treatments				
						γ-BHC		
							mg./yd. of row	
No.	Dressing	Dressing rate (g./kg.)	Seed rate	No.	Hg	oz./acre all trials	Trials 1 and 3	Trial 2
1	Undressed seed	—	L.	1	0	0	0	0
			H.	2	0	0	0	0
2	Hg only	7·0	L.	3	Hg	0	0	0
			H.	4	2 Hg	0	0	0
3	γ-BHC, 1 dose	3·5	L.	5	0	0·5	1·76	2·25
			H.	6	0	1·0	3·52	4·5
4	γ-BHC, 1 dose+Hg	3·5	L.	7	Hg	0·5	1·76	2·25
			H.	8	2 Hg	1·0	3·52	4·5
5	γ-BHC, 2 doses	7·0	L.	9	0	1·0	3·52	4·5
			H.	10	0	2·0	7·04	9·0
6	γ-BHC, 2 doses+Hg	7·0	L.	11	Hg	1·0	3·52	4·5
			H.	12	2 Hg	2·0	7·04	9·0

L.=low; H.=high.

TABLE 2. *Details of field trials 1–3, 1949*

Trial no. ...	1	2	3
Locality	University Farm, Cambridge	Easton, Hunts	Horseway, Isle of Ely
Soil	Gravel	Light Loam	Medium Black Fen
Date of drilling	23 March	14 April	14 April
Seed rates: High	26 lb./acre	22 lb./acre	26 lb./acre
Low	13 lb./acre	11 lb./acre	13 lb./acre
Pests observed	None	Pygmy mangold beetle (light)	Wireworm (light)

Small-scale field trial

In this trial (trial 4), which was sown on 28 March, a seed dressing containing 70% γ-BHC was used at four dressing rates (see Table 4). Undressed seed and seed dressed with a standard mercurial dressing (Agrosan GN) were included as controls. A high seed rate, 26 lb./acre, was used throughout, so that the deposit of γ-BHC per unit length of row would, except for treatment (1), be above normal. The plots were three rows wide and 5 yd. long, and the treatments were randomized and replicated 4 times. The seed was sown with a hand drill as in trials 1–3.

Plant counts made at the 4-leaf stage showed no significant differences between treatments. Phytocidal effects appeared in the 2-leaf stage, grew more intense throughout the 4-leaf stage and then gradually disappeared. Plants showing

TABLE 3. *Percentage plant populations in the 1949 trials, natural seed*

				Treatment effects							Mean effects						
								Hg+									
Trial no.	Count	Seed rate	Plant populations on controls	0	Hg	1 γ-BHC	2 γ-BHC	1 γ-BHC	2 γ-BHC	Sig. diff.**	0	1 γ-BHC	2 γ-BHC	Sig. diff.**	0	Hg	Sig. diff.**
1	1	High	58·3/yd.	100	131	94	106	**162**	127	±14	100	105	**105**	±8	100	**131**	±8
		Low	31·6/yd.	100	104	85	105	104	**127**	±26							
	2	High	52·7/yd.	90*	125	95	104	**144**	116	±20	93	98	100	±12	95	**121**	±11
		Low	26·1/yd.	83*	101	79	104	95	**107**	±36							
	3	High	25,500/acre	100	100	101	98	**105**	100	±6	100	**102**	99	±3	100	**100**	±2
		Low	24,900/acre	100	98	100	100	**101**	97	±6							
2	1	High	53·4/yd.	100	88	99	104	100	**119**	±18	100	110	**116**	±13	100	**104**	±14
		Low	24·7/yd.	100	103	109	113	**132**	114	±39							
	3	High	21,800/acre	100	97	98	100	**109**	101	±10	100	**108**	107	±5	100	**103**	±4
		Low	18,700/acre	100	102	111	112	**115**	114	±11							
3	1	High	78·8/yd.	100	**115**	88	84	109	99	±25	**100**	98	91	±14	100	**116**	±15
		Low	39·1/yd.	100	103	95	106	**128**	104	±50							
	2	High	70·8/yd.	90*	84	94	85	98	**99**	±22	84	**94**	86	±14	84	**91**	±11
		Low	30·9/yd.	79*	104	88	93	**119**	79	±44							
	3	High	23,000/acre	100	**103**	98	100	101	99	±7	**100**	95	97	±4	100	**102**	±3
		Low	24,100/acre	**100**	99	92	94	93	98	±7							

* The percentage plant populations in the 4-leaf stage are based on the controls in the 2-leaf stage to show changes occurring between the stages.
** Three times the standard error is taken as a significant difference between any two treatment means.

Note. The treatments giving the highest plant populations are in black in this and subsequent tables.

typical symptoms were 2–3 in. shorter in the 4- to 8-leaf stage than the controls. The cotyledons were yellowed and the rough leaves were mottled, thickened and somewhat distorted. The roots were not seriously affected. After the symptoms had disappeared, the plants appeared healthy but smaller than normal. Grading when the damage was worst gave the results shown in Table 4.

TABLE 4. *Grading of the plots in trial 4: the application of varying amounts of γ-BHC to the seed*

		Marks allotted per plot (0–5)				
		Blocks				
Seed treatment and dressing rate	Soil treatment mg. γ-BHC/yd. of row	1	2	3	4	Total marks per treatment
1. γ-BHC ½ normal	3·5	4	4	3	3	14
2. γ-BHC 1 normal	7·0	2	2	2	2	8
3. γ-BHC 1½ normal	10·5	1	1	1	1	4
4. γ-BHC 2 normal	14·0	0	0	0	0	0
5. Undressed seed	0·0	3	3	5	4	15
6. Mercury	(2 Hg)	5	5	4	5	19

The plots showing the best growth were those treated with mercury only. The plots sown with undressed seed and seed treated with γ-BHC at the lowest rate were practically indistinguishable, whereas the plots receiving higher dosages were progressively very much poorer. The date of sowing and the soil conditions were much the same as for trial 1 but here no phytotoxicity was observed, although the deposit of γ-BHC ranged up to 7 mg./yd. of row.

Demonstration strips

Two demonstration strips were laid down by each of six East Anglian beet sugar factories. Each pair had eight rows of untreated seed sown alongside eight rows of seed treated with a combined dressing containing 70% γ-BHC. The object was to test the dressing under normal farm conditions. A summary of the results is given in Table 5.

At site 2 where the soil was slightly calcium deficient, lime was applied across the strips, and separate counts were made on the limed and unlimed areas. On the limed portion at site 2, and at site 5 the combined dressing gave increased plant counts at all stages. At site 5 the large decrease in plant populations on treated and untreated plots alike at the 4-leaf stage indicates that some factor, possibly pests, was at work and that, under these conditions, the combined dressing was of considerable benefit. On the unlimed area at site 2, and also at sites 3 and 4, the combined dressing gave improved stands before singling, but decreases after singling. At sites 1 and 6, the stand was depressed by the seed dressing at all stages. Slight phytotoxicity was observed at site 6 and severe damage at site 3 both on light sandy soils. The high seed rate and the wide spacing employed at site 3 resulted in

TABLE 5. *Demonstration strips. Comparison of seed dressed with a combined dressing of γ-BHC and Hg with untreated seed*

Counts (1) At 2-leaf stage, plants/24 ft. of row. (2) At 4-leaf stage, plants/24 ft. of row. (3)* After singling, plants/4 chains. (4) Full establishment, plants/4 chains.

Strip no.	Site	Soil	Seed rate (lb./acre)	Row width (in.)	γ-BHC/yd. (mg.)	Count no.	Treated γ-BHC+Hg	Untreated (0)	Effect of treatment
1.	Welnetham, Suffolk	Medium Loam	9·5	20	3·0	(1)	182	197	−15
						(2)	195	216	−21
						(3)	298	328	−30
						(4)	300	317	−17
2.	Pymoor, Ely	Light Fen							
		(*a*) Unlimed	14	19½	4·3	(1)	351	332	+19
						(2)	280	188	+92
						(3)	315	328	−13
						(4)	321	334	−13
	Pymoor, Ely	(*b*) Limed	14	19½	4·3	(1)	353	323	+30
						(2)	284	216	+68
						(3)	330	319	+11
						(4)	331	311	+20
3.	Tatterford, Norfolk	Light Sandy Loam	19	24	7·3	(1)	574	528	+46
						(2)	549	510	+39
						(3)	244	245	−1
						(4)	208	251	−43
4.	Thorney, Peterborough	Light Fen	13	19½	4·0	(1)	296	225	+71
						(2)	306	270	+36
						(3)	299	304	−5
						(4)	236	241	−5
5.	Littlebury, Essex	Light Chalk	12·5	21	4·2	(1)	250	215	+34
						(2)	172	101	+71
						(3)	—	—	—
						(4)	315	237	+78
6.	Tunstall, Suffolk	Light Sand	14	19	4·2	(1)	247	262	−15
						(2)	262	278	−16
						(3)	332	389	−57
						(4)	326	351	−25

* In all the following tables count (3) was made at full establishment.

a greater deposit of γ-BHC/yd. of row (7·3 mg.) than at any of the other centres. The strips at site 3 were harvested and the loss in yield due to dressing was at the rate of approximately 2 tons of washed beet per acre. The symptoms were the same as those observed in the small-scale field trial but were somewhat more severe, the roots being affected as well as the foliage. The roots were discoloured and showed some constriction at soil level, reminiscent of strangles. Some plants became severed, giving rise to a serious loss of stand.

THE 1950 AND 1951 TRIALS

In 1950 and 1951 further trials and demonstration strips were laid down as in 1949. Those of 1950 were made with natural seed using a dressing in which the concentration of γ-BHC was reduced from 70 to 40%. Those of 1951 were made with seed rubbed and graded $\frac{7}{64}-\frac{11}{64}$ in. using dressings containing 20, 40 and 60% γ-BHC in the trials and 40% in the demonstration strips. In trial 14, both rubbed and natural seed were tested side by side. In all trials the treatments were randomized and replicated 4 times and the plot size was eight rows by $5\frac{1}{2}$ yd., so that each trial occupied between one-eighth and one-quarter acre. Precise drilling rates were obtained by sowing from packets containing weighed quantities of seed. In the 1950 trials, the dosage rate was 2·3 mg./yd. row, while in the 1951 trials it varied from 1·1 to 6·7 mg./yd. row according to the concentration, seed rate and dressing rate. Seed for the demonstration strips was dressed at the normal rate and the dosage of γ-BHC/yd. row varied greatly according to the farmers' seed rates but nowhere exceeded 5 mg./yd. row.

With natural seed, the normal seed and normal dressing rates were 15 lb./acre and 7 g./kg. respectively. For rubbed seed, because of its different characteristics, these rates were adjusted to give equivalent dosage of insecticide per yard of row. Thus, 10 lb./acre was taken as the normal rate for rubbed seed and the dressing rate was increased in proportion to 10·5 g./kg. seed.

Trials 5–7 were in fields that had grown sugar beet in the previous year so that there was a possibility of pygmy beetle injury. Trials 8–14 were in fields where the previous crop was other than sugar beet but were in districts where pygmy beetle was abundant. However, only slight attacks of this pest were experienced.

The results of the 1950 trials are shown in Table 6, those of the 1951 trials in Table 7 and those of the demonstration strips in Figs. 1 and 2. Dressed seed invariably gave better stands than undressed seed. In some trials, the increases in stand in the 2-leaf and 4-leaf stages (counts 1 and 2), were substantial, but these increases were largely removed by singling (count 3). The effects of seed dressing in the various trials and demonstration strips are summarized in Table 8. The best stands were most often obtained from combined insecticidal and fungicidal dressings, but the greater part of this response was most often attributable to the fungicide, and only occasionally to the γ-BHC. The responses using rubbed seed were much the same as those obtained with natural seed. In trial 14, the responses to rubbed and

TABLE 6. *Percentage plant populations in small-scale replicated trials, 1950, natural seed (untreated = 100)*

Trial no.	Count	Plant populations on controls (= 100)	Treatment effects				Mean effects				Significance			
			40 % γ-BHC	Hg	40 % γ-BHC + Hg	S.E.	40 % γ-BHC	S.E.	Hg	S.E.	All treat-ments	γ-BHC	Hg	γ-BHC × Hg
5	1	11/yd.	149	198	**261**	±9·8	**138**	±4·6	**184**	±5·6	***	***	***	—
	2	7/yd.	158	304	**327**	±22·6	**120**	±7·9	**244**	±12·3	***	—	***	—
	3	18,300/acre	119	136	**144**	±4·4	**111**	±2·6	**128**	±2·8	***	**	***	—
6	1	15/yd.	128	**143**	141	±8·6	**111**	±5·0	**124**	±5·3	*	—	**	—
	2	17/yd.	122	129	**139**	±7·6	**114**	±4·7	**121**	±4·9	*	—	**	—
	3	30,300/acre	103	**107**	105	±2·5	**101**	±1·7	**104**	±1·4	—	—	—	—
7	1	17/yd.	120	147	**161**	±6·9	**114**	±4·0	**140**	±4·5	***	*	***	—
	2	17/yd.	99	125	**152**	±7·8	**112**	±4·9	**139**	±5·5	***	—	***	—
	3	31,600/acre	98	106	**107**	±1·9	99	±1·0	**108**	±1·4	*	—	**	—
8	1	17/yd.	124	**154**	151	±6·5	**108**	±3·6	**136**	±4·1	***	—	***	—
	2	13/yd.	138	152	**158**	±9·9	**117**	±5·0	**130**	±5·2	**	*	**	—
	3	24,500/acre	108	111	**115**	±1·5	**105**	±1·0	**109**	±1·0	***	**	***	—
9	1	25/yd.	112	**130**	130	±7·8	**105**	±4·8	**123**	±5·2	*	—	**	—
	2	21/yd.	112	128	**144**	±7·7	**112**	±4·7	**128**	±5·1	**	—	**	—
	3	30,800/acre	102	103	**105**	±1·8	**102**	±1·3	**103**	±1·3	—	—	—	—
10	1	15/yd.	127	183	**204**	±9·6	**117**	±6·8	**170**	±1·4	***	*	***	—
	2	9/yd.	143	235	**261**	±8·4	**121**	±3·5	**204**	±4·9	***	***	***	—
	3	29,300/acre	110	123	**124**	±2·8	**105**	±1·8	**118**	±1·9	***	—	***	—
Combined results	1	17/yd.	124	154	**166**	±5·1	**114**	±2·8	**143**	±3·2	***	**	**	***
	2	14/yd.	123	157	**174**	±6·5	**115**	±3·6	**148**	±4·1	***	**	***	—
	3	27,500/acre	106	113	**114**	±2·3	**104**	±1·3	**110**	±1·4	***	—	***	—

* Significant at the 5 % level. ** Significant at the 1 % level. *** Significant at the 0·1 % level.

TABLE 7. *Percentage plant populations in small-scale replicated trials, 1951, rubbed seed (untreated = 100)*

			Treatment effects								Mean effects						Significance			
							Hg +													
Trial no.	Count	Plant populations on controls (= 100)	Hg	20% γ-BHC	40% γ-BHC	60% γ-BHC	20% γ-BHC	40% γ-BHC	60% γ-BHC	S.E.	20% γ-BHC	40% γ-BHC	60% γ-BHC	S.E.	Hg	S.E.	All treatments	γ-BHC	Hg	γ-BHC × Hg
11	1	24·1/yd.	118	118	110	104	113	**131**	104	±6·5	102	**106**	91	±4·1	**110**	±3·0	*	—	*	—
	2	20·0/yd.	127	121	98	97	116	**129**	123	±9·1	**104**	100	97	±5·7	**119**	±4·4	—	—	**	—
	3	33,400/acre	103	101	96	100	**105**	104	101	±4·1	**101**	99	99	±2·9	**104**	±2·1	—	—	—	—
12	1	13·1/yd.	121	**125**	118	93	124	124	118	±8·6	**113**	110	96	±5·6	**111**	±3·9	—	—	—	—
	2	12·7/yd.	116	116	105	109	**131**	115	123	±10·0	**114**	102	107	±6·6	**113**	±4·7	—	—	—	—
	3	27,500/acre	104	107	95	94	106	104	**109**	±4·4	**104**	98	99	±3·0	**107**	±2·2	—	—	*	—
13	1	9·4/yd.	**140**	126	101	97	123	129	135	±7·8	**104**	96	97	±4·6	**124**	±3·6	**	—	***	*
	2	9·1/yd.	110	**136**	113	96	136	114	120	±11·9	**129**	108	102	±8·0	**107**	±5·3	—	—	—	—
	3	30,100/acre	104	102	**108**	90	94	101	101	±5·4	96	**102**	94	±3·7	100	±2·7	—	—	—	—
14	1	24·7/yd.	98	—	107	—	—	**143**	—	±5·3	—	**127**	—	±3·8	**116**	±3·6	***	***	**	**
	2	26·7/yd.	97	—	109	—	—	**127**	—	±5·0	—	**120**	—	±3·6	**107**	±3·4	***	***	—	*
	3	28,600/acre	100	—	104	—	—	**106**	—	±2·0	—	**105**	—	±1·4	**101**	±1·4	—	*	—	—

Note. Trial 11, normal seed rate, normal dressing rate. Trial 12, half normal seed rate, normal dressing rate. Trial 13, normal and half normal seed rate, normal and twice normal dressing rate, confounded with blocks. Trial 14, normal seed rate, normal dressing rate, natural and rubbed seed.

natural seed tested side by side were so similar that only the means of both counts are given in Table 7. There was no clear or consistent difference between the three concentrations of γ-BHC in the dressings applied to rubbed seed (Table 7), except for a suggestion that the highest concentration (60 %) tended to reduce the stand below the controls (trials 11–13), but this effect was nowhere significant. The responses to seed dressing in the trial at the reduced seed rate (trial 12) were much the same as those in the trial with the normal seed rate (trial 11) and the final stand was about 18 % lower but adequate for commercial purposes. No phytotoxicity was observed in the field trials and demonstration strips in 1950 or 1951, and in this respect, the 40 % seed dressing was more satisfactory than the 70 % dressing used in 1949.

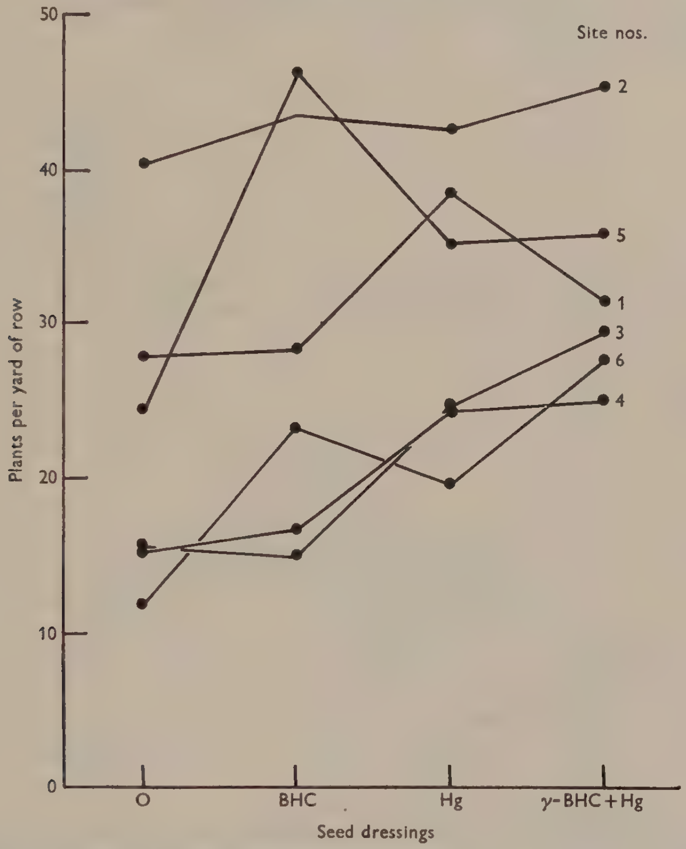

Fig. 1. The effects of seed dressings at six sites in 1950: plant populations immediately before singling. Natural seed.

In the 1950 trials, where one standard seed rate was used throughout (Table 6), the control plot counts show the variation in plant population obtained at the different sites. The seed gave 60 % germination of clusters in the laboratory and was sown at the rate of 50 clusters/yd. of row. Since on the average each cluster is capable of producing one and a half seedlings, the number of plants expected in the pre-singling stage was 45/yd. of row. A measure of the effect of adverse factors at the six trial sites is given by the extent to which the plant populations derived from undressed seed fall short of this figure. In these trials, the fungi controlled by

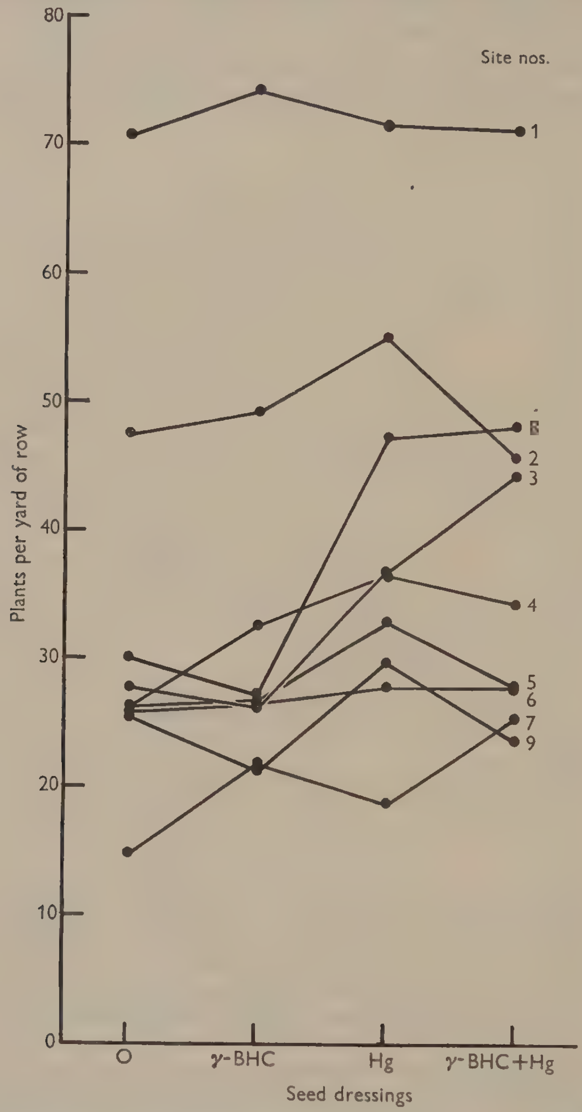

Fig. 2. The effects of seed dressings at nine sites in 1951: plant populations immediately before singling. Rubbed seed.

mercury and the pests controlled by γ-BHC were together less important than the sum of the remaining factors, most of which were probably physical in nature.

TABLE 8. *Summary of results from field trials and demonstration strips*

A. Plant population changes, field trials 1–12 and 14

	No. of trials showing			
	Significant rises	Insignificant rises	Insignificant falls	Significant falls
γ-BHC				
Two-leaf stage	5	6	2	—
Before singling	3	9	—	—
After singling	4	6	2	1
Hg				
Two-leaf stage	11	2	—	—
Before singling	8	4	—	—
After singling	6	7	—	—

B. Best individual treatments, field trials 1–12 and 14 and 1950–2 demonstration strips, pre-singling stage only

No treatment	Insecticide	Fungicide	Combined dressing	Total
1	3	7	31	42
*2 %	7 %	17 %	74 %	100 %

* Includes the results of the 1952 demonstration strips.

THE 1952 TRIALS

In 1952, fourteen replicated demonstration strips were laid down similar to those of 1950 and 1951. The seed treatments applied were as follows:

(1) No dressing
(2) Agrosan GN
(3) Panogen*
(4) Thiram 50 %
(5) γ-BHC 40 %
(6) Agrosan GN + γ-BHC 40 %
(7) Panogen + γ-BHC 40 %
(8) Thiram 50 % + γ-BHC 40 %
(9) Aldrin 40 %
(10) Thiram 50 % + Aldrin 40 %

Except for Panogen, which was applied as a liquid spray at 6 c.c./kg., all the other treatments were powder seed dressings applied at a dressing rate of 10 g./kg. The seed employed was rubbed and graded $\frac{7}{64}$–$\frac{11}{64}$ in. and was supplied from one mixed bulk. It contained 1973 clusters/oz. of seed and samples submitted to the Official Seed Testing Station produced 1·32 sprouts/cluster. The maximum number of seedlings that could be expected in the field was, therefore, 2604/oz. seed. In fact, the average number of seedlings on the control plots of the first thirteen trial sites before singling was 1329/oz. of seed, about half the expected number.

The seed was drilled with seeder units (various makes) to facilitate control of seed rates, which ranged from 7 to 12 lb./acre. The load of γ-BHC applied with the seed

* Methyl mercury dicyandiamide 2·2%.

was of the order of $\frac{1}{2}$ oz./acre or 2 mg./yd. of row. The pre-singling counts are expressed as seedlings/oz. of seed sown, which eliminates any variations in drilling that might have arisen from conditions on the sites or from the effect of the dressings themselves. Such effects are unlikely with cup-feed drills but might occur with other types, where friction between the treated seed surface and the drill mechanism is involved. Analysis of the seed rates in this series of demonstration strips and other trials, however, failed to reveal any consistent variations in the performance of cup-feed or other types of drill when drilling sugar-beet seed bearing different dressings.

TABLE 9. *Results of the 1952 factory demonstration strips. Percentage plant populations (untreated = 100)*

Sites	Plant populations on the controls (= 100)	Agrosan	Panogen	Thiram	γ-BHC	Agrosan + γ-BHC	Panogen + γ-BHC	Thiram + γ-BHC	Aldrin	Thiram + Aldrin	Significant difference
					Before singling						
1–13	1329 plants/oz. of seed	107	108	107	98	113	106	117	101	**118**	± 9
14	744 plants/oz. of seed	68	81	39	175	**248**	232	228	167	237	± 59
					After singling						
1–13	63·6 plants/chain of row	104	103	104	104	**104**	103	104	103	104	Insignificant
14	47·4 plants/chain of row	90	102	77	**156**	155	154	145	152	153	± 18

The results from the demonstration strips are presented in Table 9. At thirteen out of the fourteen sites, the responses to seed dressing were fairly consistent and not very large. At the fourteenth site, treatment responses were very different and for this reason are given separately. Over all sites, including the fourteenth, the error terms in the statistical analysis of the results were of the same order so that a single estimate of pooled error was applicable throughout. The means of counts from the first thirteen sites showed significant differences in the pre-singling stage, the best stands being given by combined dressings. After singling there were no significant differences, although seed dressing gave a consistent increase in stand of 3–4% over the undressed controls. At the fourteenth site, there was an unexpected attack by wireworms, and large significant responses were obtained to both insecticides before and after singling, which brings out the value of insecticidal seed dressings as an insurance. There was insufficient evidence upon which to assess the respective merits of γ-BHC and aldrin.

Effect of seed dressing upon germination

In 1950, samples of natural seed were submitted to the Official Seed Testing Station for germination tests immediately after dressing and after storage for 7 months. At the end of this period, there had been no material change in the germinating power of the seed, nor was there any significant effect due to dressing. In 1952, more elaborate germination tests were made with the rubbed seed employed in the fourteen replicated demonstration strips. Samples of treated and untreated seed were stored in paper packets for 0, 3, 6, 9, and 12 months, and were submitted to the Seed Testing Station as before. The results obtained are set out in Table 10.

Table 10. *Summary of results of germination tests. Effects of seed dressing and storage on germination of rubbed seed*

	Percentage germination of clusters					Significant differences
Fungicides ...	O	Agrosan	Panogen	Thiram		
	84·6	84·4	82·2	—		±2·6
	84·4	—	—	84·5		±2·0
Insecticides ...	O	γ-BHC				
	83·5	83·8				±1·7
	O	Aldrin				
	85·5	85·5				±2·6
Time stored in months ...	O	3	6	9	12	
	85·0	83·2	84·5	82·8	84·5	±2·6
	Sprouts per 100 clusters					
Fungicides ...	O	Agrosan	Panogen	Thiram		
	129	134	132	—		±8·0
	132	—	—	132		±6·1
Insecticides ...	O	γ-BHC				±5·1
	134	129				
	O	Aldrin				
	134	137				±8·0
Time stored in months ...	O	3	6	9	12	
	139	132	128	129	134	±8·0

Although certain individual treatments were just significantly different from others, the mean effects of the various dressings upon percentage germination of the clusters were all insignificant. γ-BHC caused a slight decrease in the number of sprouts produced per 100 clusters but this effect only just reached significance. There was also a slight, significant deterioration in sprouting power due to storage, although this effect was irrational. It seems clear that the seed dressings used had no serious effect upon the vitality of the seed when stored in the manner described and no complications arose in the germination tests similar to those reported by Jameson & Callan (1951) for cereals dressed with combined dressings.

THE PESTS CONTROLLED BY INSECTICIDAL SEED DRESSING

Where pest activity is at a low level, as in most of the trials, the samples taken for the assessment of pest injury are composed largely of healthy seedlings together with a few injured seedlings which may or may not have survived had they not been removed. Seedlings that have already succumbed soon disappear without leaving any indication of the cause of death. For these reasons it proved difficult to form an accurate picture of the pests causing the small responses to insecticidal dressing in most trials. The general impression gained was that most of this small improvement in stand was due to the control of wireworms and to the destruction or deterrence of other marauding insects making chance attacks upon the seed in the early stages of germination. Pygmy mangold beetle populations were high at trials 2 and 5–7. At trials 2, 6 and 7 there were no large responses from seed dressing with γ-BHC. Responses were greater at trial 5, but only very partial control was obtained. Examination of plant samples was inconclusive. No clear-cut control of pygmy mangold beetle appears to be achieved by the use of γ-BHC dressings, possibly because the feeding of the beetles is generally too high up on the plant, above the zone made lethal by the dressing. Attacks by other seedling pests were too slight for any conclusions to be drawn about the effectiveness of seed dressing against them.

CONCLUSIONS

Seed dressings containing from 35 to 45 % γ-BHC are safe for general use on rubbed and natural sugar-beet seed. For rubbed seed, the dressing rate may be increased if desired from the usual rate of 7 g./kg. up to 10 g./kg. of seed to offset the smaller quantity normally sown. Dressings containing higher concentrations of γ-BHC (up to 60 %) could be used in most seasons and on most soils without undue risk of damage provided normal seed rates were employed.* The soils on which phytotoxicity is most likely to occur are light sands and light black fen, especially in seasons that are dry and warm during early growth. Dressing with γ-BHC does not appear to affect germination of seed seriously, even after periods of storage of up to 1 year.

Because of the phytotoxic nature of γ-BHC, seed dressing with this insecticide is best done by the seed merchant who is better able to ensure correct dosage and thorough mixing. On the Continent, where farmers sometimes dress seed themselves, trouble arises from overdressing, maldistribution of dressing, and from double dressing where the seed has first received an ordinary organic mercury treatment in powder form.

In ordinary beet fields where no special pest problems are anticipated, an excess of beet seed of high germinating power is sown. Less than half the potential number of seedlings appears above ground and a further small fraction dies before singling. Estimation of the part played by pests in this heavy loss was not possible until the appearance of γ-BHC as an effective soil insecticide. When this is applied to normal fields as a seed dressing, the average increase in plant population is small. The results of the field trials herein described may be expressed in round figures as follows: expected number of seedlings 100, losses due to insects 5, losses due to fungi 10, losses due to other causes, mainly physical 55, seedlings surviving at singling time 30. The relative loss from pests and fungi is reduced still further by singling. The value of seed dressing, however, is not entirely made clear by average figures. Where, for reasons unknown, wireworms or fungi are unusually active, seed dressings give excellent returns. They are cheap and convenient to apply and the money spent on them is best regarded as an insurance, especially where low seed rates of natural or processed seed are used to ease the labour problems of singling. When heavy wireworm attack is expected after ploughing grass or on soils where the arable wireworm problem is acute, combined dressings should be used with an increased seed rate. The risks of damage from the heavier deposit of γ-BHC are quite overshadowed by the improvements in stand obtained. Care should be taken to avoid over-deep drilling which may allow wireworms to feed upon the hypocotyl above the zone of influence of the dressing.

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* In 1954, conditions during the seedling stage of the sugar beet crop appear to have been more conducive to γ-BHC phytotoxicity than in the five preceding years. Marked injury occurred in one or two localities where a 40% γ-BHC seed dressing had been employed.

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PROTECTION OF SORGHUM AGAINST SOIL FUNGI, SOIL PESTS AND COVERED SMUT BY COMBINED INSECTICIDE–FUNGICIDE SEED DRESSINGS

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Treatment of sorghum (*Sorghum vulgare*) seed with fungicide dressings controlled covered smut (*Sphacelotheca sorghi*), the major disease of sorghum in the Sudan, and usually resulted in appreciably better stands. In the Sudan Gezira, powder dressings containing 20–40 % γ-benzene hexachloride (γ-BHC) applied at the rate of 1 g./lb. of seed effectively protected sorghum seedlings from root attack by chafer grubs (*Schizonycha* sp.) and significantly increased stands and yields. No certain evidence of this effect was obtained with dolichos bean (*Dolichos lablab*) or groundnut (*Arachis hypogaea*), and the different responses shown by these plants as compared with sorghum are attributed largely to differences in seed germination and root system. An application rate of 0·08 % γ-BHC/seed was sometimes slightly but appreciably phytotoxic to sorghum in the field and more markedly so in greenhouse experiments. A powder containing 25 % thiram and 20 % γ-BHC applied at the rate of 1 g./lb. of seed is recommended as the standard seed dressing for sorghum in the Sudan.

It is only during recent years that chafer grubs have come to the fore as potentially serious pests in the Sudan Gezira. Previously, chafer larvae have been reported as feeding on cotton roots and thereby causing wilting (Cowland, 1933, p. 96), as causing appreciable damage to young dolichos bean (Bedford, 1939, p. 59), and as responsible for severe loss of stand in late-sown sorghum (Bedford, 1936, p. 75). At the Gezira Research Farm poor stands of dolichos frequently occur when this plant is sown on land cropped to sorghum in the previous season.

This paper summarizes research on seed dressings for sorghum (*Sorghum vulgare* Pers.) carried out in the Sudan in 1948–53. Sorghum is the major food crop, and several million acres are cultivated annually under irrigation or rain throughout the country. Seed treatment is a method of disease control well suited to native agriculture in that it is a simple, inexpensive operation not requiring elaborate apparatus or specialized technical skill. Except where otherwise stated, these experiments were carried out in the Gezira, the extensive flat clay plain lying between the Blue and White Niles south of their junction at Khartoum; in this plain large acreages of rain-grown and irrigated sorghum are cultivated. Sowing was in spaced holes, made by sowing stick, with ten seeds per hole as is the usual method in the Sudan. Throughout this paper 'γ-BHC' denotes the γ isomer of benzene hexachloride.

COMBINED FUNGICIDE-INSECTICIDE SEED DRESSINGS ON SORGHUM

Preliminary plot experiments

In plot experiments sorghum seed treated with combined fungicide-insecticide powders produced a markedly better stand than untreated seed or that treated with fungicide only. This occurred with irrigated sorghum planted on ridges and on the flat and with rain-grown sorghum, all sown in mid-July (Table 1).

TABLE 1. *Results of seed treatment of sorghum, var. Feterita Managil: preliminary trial*

	% stand			
	Irrigated			
Seed dressing and application rate	Ridge	Flat	Rain grown	Average
(1) None	84·5	54·7	48·0	62·4
(2) Sulphur, 1:150	91·6	65·8	49·7	69·0
(3) Copper carbonate, 1:300	94·9	66·3	64·7	75·3
(4) Spergon, 1:500	94·4	66·6	62·8	74·6
(5) Phygon, 1:500	94·4	80·1	50·8	75·1
(6) 10% thiram + 40% γ-BHC, 1:500	98·2	86·5	66·2	83·6
(7) 50% thiram + 40% γ-BHC, 1:500	98·9	83·6	80·9	87·8
(8) O-mercurial + 20% γ-BHC, 1:500	97·6	89·9	75·9	87·8
Average, fungicide only (2–5)	93·8	69·7	57·0	73·5
Average, fungicide + γ-BHC (6–8)	98·2	86·7	74·3	86·4
Percentage increase with γ-BHC	4·7	24·4	30·4	19·8

Note. Spergon = tetrachloroparabenzoquinone; Phygon = 2:3-dichloro-1:4-naphthoquinone. thiram = tetramethylthiuram disulphide (TMTD); γ-BHC = γ-isomer benzene hexachloride; O-mercurial = organo-mercurial containing equivalent of 1% mercury.

Seed treatment with fungicide increased stands (measured as percentage of good holes to total holes sown) in all three plots by some 18%, sulphur being the least effective. Incorporation of 20% or 40% γ-BHC increased stands by about 20% as compared with fungicide only, and by nearly 40% as compared with untreated seed. As would be expected, ridge-irrigated sorghum receiving near-optimum moisture conditions produced the best stand. It also showed least response to γ-BHC.

In a second, replicated plot experiment with ridge-irrigated sorghum details of percentage stand, number of surviving seedlings, number of heads produced and weight of straw (stalks and leaves) were recorded (Table 2). Stand and seedling counts were made 3 weeks after sowing; the sowing date being mid-July.

Fungicide-treated seed gave slightly but not significantly better stands and straw yields than untreated. Seed dressed with fungicide-γ-BHC powders produced about 38% more plants, 9% better stand, 20% more heads and 15% more straw than that treated with fungicide only. These well-marked and significant increases were almost certainly due to the protective action of the insecticide against chafer grubs which attacked the young sorghum seedlings, as discussed below.

TABLE 2. *Results of seed treatment of sorghum, var. Feterita Managil*

Seed dressing and application rate	% Stand	No. of seedlings	No. of heads	Wt. straw, kg.
(1) None	84·8	7854	12141	755·5
(2) Sulphur, 1:300	87·7	9115	11787	754·5
(3) Copper carbonate, 1:300	85·2	7932	12202	768
(4) Spergon, 1:500	88·3	9611	12856	792
(5) Phygon, 1:500	92·4	10125	12040	816
(6) 50% thiram, 1:500	85·6	9795	12590	794
(7) 50% thiram, 1:1000	85·7	8458	10111	728·5
(8) 10% thiram+40% γ-BHC, 1:500	94·9	12978	14241	888
(9) 50% thiram+40% γ-BHC, 1:500	95·7	12890	14740	928
(10) O-mercurial+20% γ-BHC, 1:500	95·5	12180	14066	864
Minimum significant difference ($P=0·05$)	8·75	1905	1748	114·3
Average, fungicide only (2–7)	87·5	9173	11931	775·5
Average, fungicide+γ-BHC (8–10)	95·4	12683	14349	893·3
Percentage increase with γ-BHC	9·0	38·3	20·3	15·2

Field trials

Detailed observations were made on a field of sorghum (var. Feterita 1931) divided into twenty quarter-acre strips, half of which were sown with seed dressed with 50% thiram powder and half with seed dressed with 50% thiram-20% γ-BHC powder at 1 g./lb. of seed. Wilting of seedlings grown from the fungicide-treated seed became apparent 2–3 weeks after sowing. The leaves of affected seedlings became limp, brown and dry as death occurred. Seedlings grown from fungicide–γ-BHC-treated seed were rarely affected and gave good even stands. Chafer grubs of the genus *Schizonycha* (Melolonthinae) were present in the soil throughout the field and were attacking the young sorghum roots just below soil level, thus causing wilting and death of the young plants. Affected plants tended to occur in fairly well-defined patches, and bare patches several yards in diameter with hardly a surviving plant were not infrequent in strips sown with fungicide-treated seed. Seedlings grown from fungicide-γ-BHC-treated seed were rarely attacked, although chafer grubs were present in their vicinity—presumably they were repelled by the insecticide. The field was fairly uniform and the stand and yield differences summarized in Table 3 are to be attributed almost entirely to chafer attack. Growth estimates were made 2 months after sowing; percentage stand was that at time of harvesting.

In the most severely attacked strip (no. 4) very poor growth with reduction in stand and in yield of grain and straw by almost 50% resulted from grub attack. Considering all strips it is evident that addition of 20% γ-BHC to the seed dressing produced very markedly improved stand and increased grain and straw yields by some 25–30%.

Replicated experiments showed that even where no chafer grub damage was evident the inclusion of 20% γ-BHC in the seed dressing usually resulted in slightly but appreciably better stands. Detailed results are too lengthy for inclusion in this paper. In one experiment monthly sowings were made from mid-July, the normal date for Gezira sorghum, to mid-November. Incorporation of 20–40 % γ-BHC in the seed dressing significantly increased percentage survival (i.e. number of surviving seedlings as percentage of total number of seeds sown) by amounts ranging from 7·6 % in the November sowing to 25·8 % in that sown in October, with an average increase of about 16 % for all sowings. Yields were also slightly but not usually significantly increased. Fungicide-treated seed gave slightly better stands and yields than untreated seed. No chafer grubs were found but these are not easily detected if present only in small numbers. There was no evidence of other soil insects being involved.

TABLE 3. *Results of seed treatment of sorghum, var. Feterita 1931*

Seed dressing: 50 % thiram					Seed dressing: 50 % thiram + 20 % γ-BHC				
Strip	Growth	% Stand	Grain (ard./fdn.*)	Straw (tons/fdn.*)	Strip	Growth	% Stand	Grain (ard/fdn.*)	Straw (tons/fdn.*)
1	Fair	91·5	5·70	3·12	11	Fair	98·2	5·42	2·74
2	Fair	82·5	4·31	2·05	12	Fair	98·4	5·51	2·86
3	Poor	62·2	4·14	1·86	13	Fair	97·9	5·51	2·79
4	Bad	52·3	2·62	1·45	14	Fair	98·9	5·59	2·86
5	Poor	79·0	4·18	1·94	15	Good	98·8	5·87	3·04
6	Fair	85·0	5·64	2·65	16	Good	96·4	5·91	2·98
7	Fair	81·2	5·67	2·33	17	Fair	97·2	5·17	2·50
8	Fair	90·3	5·46	2·49	18	Good	97·5	5·32	2·56
9	Poor	68·4	4·16	2·04	19	Good	98·5	5·95	3·21
10	Poor	53·6	3·51	1·89	20	Good	98·3	6·09	2·71
Average		74·6	4·54	2·18			98·0	5·63	2·82

* 1 ardeb = approx. 332·6 lb.; 1 feddan = 1·038 acres.

In these experiments maximum mortality due to chafer grub attack usually occurred some 10–21 days after sowing, but in poor soil plants up to a month or so old were sometimes killed. The sorghum seedling appeared to be vulnerable until the establishment of its surface adventitious roots, about 3–4 weeks after sowing, after which it became far less liable to fatal attack. Seed dressings containing γ-BHC protected the seedlings during this early vulnerable stage by repelling the chafer grubs. Factors promoting vigorous seedling growth tended to reduce chafer damage; in one experiment sown in poor soil addition of 20 % γ-BHC to the seed dressing increased percentage survival from 35 to 68 % in unmanured subplots and from 58 to 89 % in subplots which had received 400 lb. of ammonium sulphate per acre.

Some of the poor stands of sorghum frequently seen in the Gezira and usually attributed to poor seed or over-watering are undoubtedly caused by chafer grubs, and further investigation will almost certainly indicate that this pest is responsible for widespread damage to this crop.

Rate of application

Seed dressings at lower application rates of γ-BHC were also tried, but in a small November-sown trial in heavily infested soil 0·02 % γ-BHC/seed was inadequate (Table 4).

TABLE 4. *Results of seed treatment of sorghum, var. Feterita Managil, with 0·02–0·08 % γ-BHC/seed*

Seed dressing and application rate	% γ-BHC/seed	% Survival* after		Fresh wt. of plants after 2 months (kg.)
		21 days	2 months	
None	0·0	27·6	19·7	2·09
O-mercurial 1:500	0·0	26·3	17·5	1·71
O-mercurial + 20 % γ-BHC, 1:1000	0·02	69·5	54·9	6·01
O-mercurial + 20 % γ-BHC, 1:500	0·04	85·0	79·5	12·56
O-mercurial + 40 % γ-BHC, 1:1000	0·04	76·9	71·3	9·87
O-mercurial + 40 % γ-BHC, 1:500	0·08	65·8	64·6	11·13

* % survival = percentage of surviving seedlings to number of seeds sown.

Differences in stand were striking. Subplots sown with untreated or fungicide-treated seed were sparse with numerous dead plants; those which survived were stunted and would probably never have produced heads. Seed treated with fungicide-γ-BHC powders gave regular stands, and most of the plants were healthy and forming heads. Seed treated at the lowest application rate (0·02 % γ-BHC/seed) produced stands noticeably inferior to those from the higher dosages but superior to those from fungicide-treated seed. These differences were reflected in the fresh weight of the plants after 2 months. In this experiment and in some others an application rate of 0·08 % γ-BHC/seed was slightly but appreciably phytotoxic; in other trials no such effect was apparent. Such differences were probably due to differing soil and climatic factors.

Phytotoxicity

Phytotoxicity of γ-BHC to sorghum was more pronounced in pot experiments (Table 5).

TABLE 5. *Results of seed dressings on sorghum, var. Feterita Managil, in pots*

Application rate	% mercury/seed	O-mercurial only		O-mercurial + 20 % γ-BHC		O-mercurial + 40 % γ-BHC	
		γ-BHC/seed	% germn.	γ-BHC/seed	% germn.	γ-BHC/seed	% germn.
1:1000	0·001	0·0	93·4	0·02	92·6	0·04	94·4
1:500	0·002	0·0	99·4	0·04	95·0	0·08	51·8
1:250	0·004	0·0	98·4	0·08	56·0	0·16	9·8

Note. % germination = % normal seedlings, excluding seed which had germinated slightly and then died.

The organo-mercurial showed no phytotoxicity, even at 0·004 % mercury/seed. Application rates of 0·02 and 0·04 % γ-BHC/seed did not affect germination and 0·08 % γ-BHC/seed produced slightly underdeveloped and abnormal seedlings. 0·16 % γ-BHC/seed was markedly phytotoxic, very few normal seedlings being produced. In the field it is possible that under conditions of heavy grub attack the greater repellent effect of 40 % γ-BHC more than compensates for its slight initial toxicity to sorghum seedlings.

Observations in the central rainlands

This area comprises a belt of land south of the Gezira and running across the Sudan from west to east. Rainfall is adequate for widespread cultivation of sorghum and other crops and large-scale mechanized sorghum production is contemplated. Limited field trials in this area have shown that better stands and yields of sorghum are to be expected when the sowing seed is treated with combined fungicide-γ-BHC powders. In a replicated trial (var. Feterita Maatuk) addition of 20–40 % γ-BHC increased stands by some 40–50 % and grain and straw yields by 30–40 %. Fungicide seed dressings, especially those containing thiram, resulted in slight stand improvement as compared with untreated seed. Subsequent observations suggest that in some cases at least the γ-BHC in the seed dressing repels small ants (*Pheidole rugaticeps* Em.) which tend to consume or carry away seed not so treated. This is likely to be of considerable importance in mechanically sown sorghum where even stands are very desirable.

CONTROL OF COVERED SMUT OF SORGHUM BY SEED TREATMENT

Covered smut caused by *Sphacelotheca sorghi* (Link) Clinton can cause heavy losses to sorghum throughout the Sudan, and fields showing 50–60 % smutted heads are not uncommon in some areas; these are responsible for grain losses amounting to many thousands of tons annually. Fortunately the disease can be reduced to negligible proportions by dusting the sowing seed with a suitable fungicide dressing.

Replicated field trials were carried out on irrigated and, occasionally, rain-grown sorghum (var. Feterita Managil) in the Gezira and on rain-grown sorghum (var. Feterita Maatuk) in the central rainlands, the sowing seed being inoculated with spores of *S. sorghi* collected from diseased heads of the same variety at the end of the previous season. Experiments indicated that an application rate of spores/seed of 1 : 100 or 1 : 1000 by weight resulted in moderately heavy smut infection, 1 : 10,000 gave less infection and the very low rate of 1 : 100,000 produced a smaller but still appreciable percentage of smutted heads. Infection was closely associated with soil conditions at and just after sowing, and was favoured by cold wet soil such as occurs after prolonged heavy rain. In irrigated sorghum plots infections varying from 5 to 25 % smutted heads were obtained. In some cases the primary heads were only slightly smutted although the secondary heads, formed after cutting back and allowing fresh growth to take place, showed considerable infection. Since *S. sorghi* is known to attack only the young seedling soon after germination it seems that in such cases the pathogen had invaded the plant but had not reached the flowers by the time the heads were formed. Rapidly growing plants thus tend to develop fewer smutted heads than those of less vigorous growth.

For several years the standard seed dressing for control of covered smut of sorghum in the Sudan has been copper carbonate used at the rate of 1·5 g./lb. of seed. Recent investigations have indicated that powders containing 25 % or 50 % thiram used at about 1 g./lb. of seed are somewhat more effective, and are stated to be virtually non-poisonous, an important factor since treated seed may be eaten by human beings or fed to stock. In addition, Agrosan GN (organo-mercurial), Spergon and Phygon (organic fungicides reported as non-poisonous) gave excellent control at 1 g./lb. Copper carbonate at 1·5 g./lb. and powdered sulphur at 3 g./lb. were slightly less effective, whilst sulphur at 1·5 g./lb. gave significantly poorer control.

In these plot experiments sowing conditions were favourable, and many of the seed dressings tested gave adequate control of covered smut, especially so since this disease does not spread from plant to plant in the field. The Feterita varieties used were probably not highly susceptible to *S. sorghi*. It is likely that with highly susceptible sorghums grown under climatic and soil conditions conducive to smut development some of the less effective seed dressings described above would give inadequate control. Addition of 20 and 40 % γ-benzene hexachloride to thiram and organo-mercurial seed dressings did not adversely affect their effectiveness against covered smut. It is recommended that the standard seed dressing for sorghum in the Sudan should be a powder containing 25 % thiram and 20 % γ-BHC, used at the rate of 1 g./lb. of seed. In addition to giving protection against covered smut and certain soil pests this dressing should also discourage storage pests.

Combined fungicide-insecticide seed dressings on dolichos and groundnut

In small trials effects, similar to those described above for sorghum, resulted from the use of combined fungicide-γ-BHC seed dressings on the wild *Sorghum purpureo-sericeum* Aschers & Schweinf and, to a less well-defined extent, on maize (*Zea mays* L.) but not on dolichos bean (*Dolichos lablab* L.), groundnut (*Arachis hypogaea* L.) and Egyptian cotton (*Gossypium barbadense* L.). With the last three crops γ-BHC seed treatment usually gave slightly but not significantly improved stands and yields, and further detailed investigation is needed. Chafer grubs can cause severe damage to dolichos and groundnut, and there was no indication in these experiments that γ-BHC seed treatment protected these plants from attack. This is probably because with these plants the seed bearing the dressing is carried above ground during germination, whereas in sorghum it remains in the soil and the latter plant develops numerous surface roots which are probably less vulnerable to grub attack than the tap-root systems of dolichos and groundnut. Thus dolichos and groundnut plants are often killed by chafer grubs a month and more after sowing, but sorghum plants of that age only rarely succumb; in one groundnut plot where grub attack became apparent some 6 weeks after sowing plants grown from γ-BHC-treated seed were not appreciably less affected than others.

The assistance of Messrs Imperial Chemical Industries Ltd., Plant Protection Ltd and United States Rubber Co. in supplying samples of seed dressings is gratefully acknowledged. My thanks are also due to my colleague Mr D. G. Pollard with whom the entomological aspects of this work were discussed.

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FIELD STUDIES ON THE INFLUENCE OF WEATHER ON EGG-LAYING BY THE CABBAGE ROOT FLY, *ERIOISCHIA BRASSICAE* BCHÉ. II

By MARY MILES

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(With 3 Text-figures)

Observations on the intensity and duration of egg-laying by *Erioischia brassicae* Bché were continued in 1953 on the same site as in 1952. Eggs were first observed on 21 April and the spring peak period of egg-laying lasted from 3 to 30 May with a daily average of 40 eggs/plant. The mean total in spring 1953 was 1287 eggs/plant. Summer peaks occurred on 1–20 July (average 17 eggs/day/plant) and 5–11 August (average 21 eggs/day/plant).

In the field the threshold of reproduction was approximately 60° F. The rate of egg-laying was highest at 65–70° F. and it declined during prolonged periods of higher temperatures.

The annual cycle of generations, as shown by egg counts and confirmed by the examination of puparia, consisted of two generations and a partial third. The spring generation developed without a prolonged diapause, and there was complete emergence during the summer. The overwintering puparia came from the second and third generations of larvae.

DURATION AND INTENSITY OF EGG-LAYING IN SPRING 1953

The plot used in 1952 was planted again in March 1953 with twenty-four cauliflower seedlings at 2 ft. apart. Soil from the bases of alternate plants was removed each morning and eggs of *E. brassicae* were extracted by flotation; they were recorded as having been laid on the previous day. The duration of the spring egg-laying period and the mean number of eggs per day per plant are shown in Fig. 1.

Eggs were first found on 21 April, but the rate of egg-laying was low until the beginning of May. The peak period of egg-laying began on 3 May when the rate increased to twenty-seven eggs per plant, and continued until 30 May; in the first half of June the rate declined to an average of nine eggs per day per plant. The highest rate occurred on 11 May when an average of seventy-eight eggs per plant was recorded. The mean daily rate during the peak period (3–30 May) was forty eggs per plant, an increase of nearly 70% over the rate for 1952 (Miles, 1953). In 1953 the peak period lasted 28 days, a week longer than that of 1952. By 14 June the mean total was 1287 eggs per plant, more than twice as many as in the same period of the preceding year.

INFLUENCE OF WEATHER ON SPRING EGG-LAYING

The peak period of egg-laying began suddenly when the maximum day temperature rose from 47 to 65° F. The highest rates of egg-laying occurred when the daily maxima were between 65° and 70° F. and there was a temporary fall to six eggs per plant on 15 May when the maximum temperature dropped to 57° F. In 1953 temperatures of over 70° F. were registered for 7 consecutive days (20–26 May) compared with fourteen in 1952 (15–28 May) and the rainfall (1·66 in. in May 1953) was 40% higher and more evenly distributed; it is suggested that this cooler, more rainy weather was the most important factor contributing to the longer period and greater intensity of egg-laying in 1953.

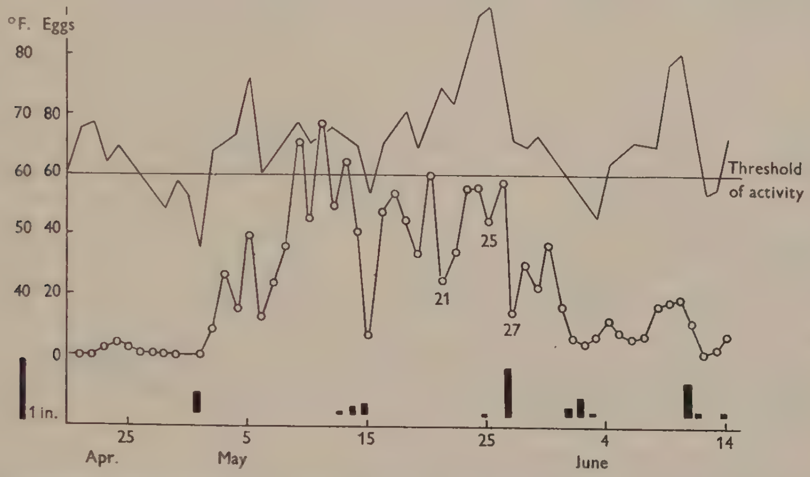

Fig. 1. Average daily rate of egg-laying by *E. brassicae* on twelve cauliflower plants in spring 1953. o—o—o, number of eggs; ——, maximum day temperature; ▮, rainfall (shown for May and June).

EGG-LAYING IN SUMMER AND AUTUMN

On 15 June the plants on the plot were cut off at soil level and another twenty-four seedling cauliflowers were set out. Eggs from alternate plants were counted daily in June and July, 3 times a week in August and September, and twice weekly in October. Fig. 2 shows the average rate of egg-laying on twelve plants from mid-June to the end of September. As in 1953 the numbers of eggs in October were so low as to be negligible.

Eggs were found on the plants the day after setting out but the numbers were generally small until 30 June. Summer peaks occurred from 1 to 20 July and from 5 to 11 August when the rates were respectively seventeen and twenty-one eggs per day per plant. As in 1952, the daily rate of egg-laying was much lower in summer than in spring. The numbers of eggs recorded during the two summer peaks were 4076 and 1815, compared with 13,475 recorded for the spring peak period. The low rate of egg-laying in summer was not associated with lack of flies because the over-wintering generation on the observation plot in 1952 was thirty-three viable pupae while counts showed that 251 flies had emerged during the summer of 1953. It appeared to be the effect of summer weather conditions. The accumulated hour-degrees above 60° F. in July and August were respectively 12 and 70% higher than in May. It is suggested that these higher temperatures promoted great activity in the flies, and this shortened their lives (Mellanby, 1938) and curtailed egg-laying. This effect was less marked in 1953 than in 1952 when temperatures were higher and rainfall less.

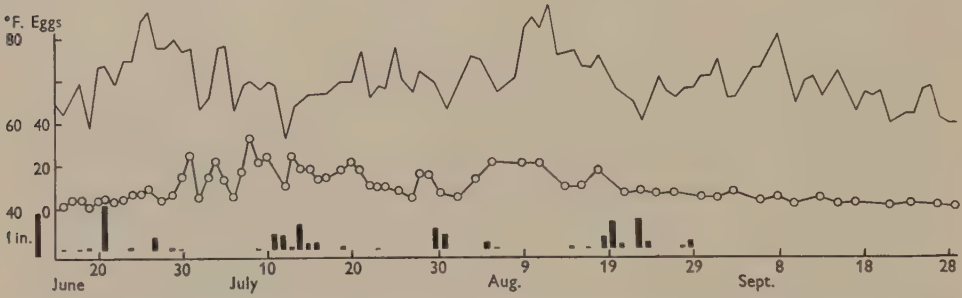

Fig. 2. Average daily rate of egg-laying by *E. brassicae* on twelve cauliflower plants in summer 1953. o—o—o, number of eggs; ——, maximum day temperature; ▮, rainfall (shown for July and August).

THRESHOLD OF REPRODUCTION

From observation in 1952 and 1953 it was apparent that when the maximum daily temperature was less than 60° F. egg-laying practically ceased. The threshold of reproduction (Rivnay, 1952) (in the case of *E. brassicae*, the threshold of oviposition) in the field was therefore approximately 60° F. In the laboratory the temperature necessary to stimulate egg-laying in captive flies was about 70° F. This discrepancy was explained by the fact that out of doors the flies fed and basked in direct sunlight while the thermometer recording the temperature was screened. In captivity the flies were practically immobilized by temperatures below 60° F. and the pre-oviposition period was indefinitely extended; feeding and mating took place at room temperatures of 65–70° F., and it was only at somewhat higher temperatures that large numbers of eggs were laid.

The observed temperature of 60° F. for the threshold of reproduction in the field is an artificial estimate of the temperature of the habitat of the flies, but it is a useful guide to the conditions under which egg-laying takes place (Miles, 1951).

ANNUAL CYCLE OF GENERATIONS

The annual pattern of egg-laying in 1953 (Fig. 3) indicated that the annual cycle of *E. brassicae* was composed of three generations, and an attempt was made to confirm this by observations on the puparia.

The twelve undisturbed cauliflowers of the first crop were lifted on 4–13 August, each with an 8 in. cube of soil. From them a total of 336 puparia were collected: of these, 133 had been parasitized; four contained dead pupae; flies had emerged from 196, and three still contained viable pupae, two almost ready to emerge. These records showed that the generation arising from eggs laid in spring had developed without a prolonged pupal diapause and that emergence of the adults was practically complete by mid-August.

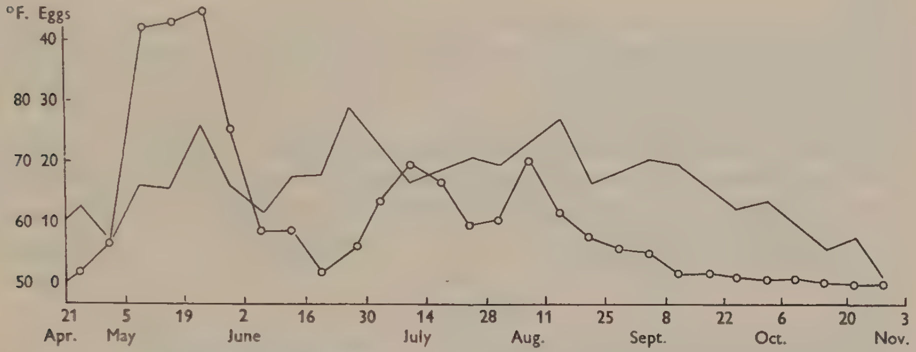

Fig. 3. Average daily rate of egg-laying by *E. brassicae* for weekly periods in 1953; o—o—o, number of eggs; ——, maximum day temperature

On 30 November–7 December the twelve undisturbed cauliflowers from the second crop were examined and 218 puparia of *E. brassicae* were collected. Of these, forty-two contained parasites or dead pupae; flies had emerged from fifty-five, and the remaining 121 appeared to contain viable pupae though some were newly formed and might later prove to be parasitized. The empty puparia at the roots of the second crop of cauliflowers showed that there had been a second generation of flies, but the small number suggested that emergence from second generation puparia was only partial and that some pupae passed directly into the overwintering diapause.

This annual cycle of two generations with a partial third agrees with the findings of Smith (1927) and is in harmony with the annual pattern of egg-laying observed in 1952 and 1953.

DISCUSSION AND CONCLUSIONS

In 1953 the economic importance of *E. brassicae*, as shown by the numbers of eggs laid on plants under observation, again declined in the succeeding generations. Differences in the intensity and duration of the spring attacks in 1952 and 1953 were related to differences in weather conditions, the lower temperatures and higher rainfall in 1953 enabling the flies to live longer and lay more eggs. Spring weather seemed generally more suited to the flies than summer weather. Marked differences between the summer weather of 1952 and that of 1953 resulted in great differences between rates of egg-laying in the respective years. In July 1952 the average maximum daily temperature was 77° F. and the total rainfall 0·55 in.; the corresponding figures for July 1953 were 69° F. and 2·46 in. In 1952 the July peak of egg-laying was small, and the August peak associated with the partial third generation was insignificant. Lower temperatures and more rain in July 1953 enabled the second generation to maintain a relatively high rate of egg-laying, which resulted in a more numerous third generation and a significant peak of egg-laying in August.

The observations on the annual pattern of egg-laying in 1952 and 1953 suggest that the summer generations of *E. brassicae* have relatively little importance and that the risk of damage to susceptible crops set out after the middle of May is negligible.

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PROVISIONAL LISTS OF HOST PLANTS OF SOME ROOT EELWORMS (*HETERODERA* SPP.)

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In host-range tests carried out at Cambridge, 1951–3, beet eelworm was found to parasitize nearly all Cruciferae tested, while other hosts were found amongst the Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Labiatae, Phytolaccaceae, Polygonaceae, Scrophulariaceae and Tropaeolaceae. Cabbage eelworm parasitized nearly all Cruciferae tested, and a few Labiatae. Hosts of *Galeopsis* and clover eelworms were found amongst the Caryophyllaceae, Labiatae, Leguminosae, Polygonaceae and Scrophulariaceae. Potato eelworm cysts were found only on *Lycopersicum* and *Solanum*; carrot eelworm only on *Daucus*; and hop eelworm only on *Urtica* (Urticaceae) and *Cannabis* and *Humulus* (Cannabinaceae). Pea eelworm appeared to parasitize only the genera *Lathyrus*, *Lens*, *Pisum* and *Vicia* (Leguminosae), while hosts of oat eelworm appeared to be distributed more or less at random within the Gramineae.

Introduction

The host ranges of nine eelworm species and varieties were investigated, namely:

Heterodera schachtii Schmidt, 1871	Beet eelworm
H. cruciferae Franklin, 1945	Cabbage-root eelworm
H. schachtii var. *trifolii* Goffart, 1932	Clover-root eelworm
H. schachtii var. *galeopsidis* Goffart, 1936	*Galeopsis*-root eelworm
H. rostochiensis Wollenweber, 1923	Potato-root eelworm
H. carotae Jones, 1950	Carrot-root eelworm
H. humuli Filipjev, 1934	Hop-root eelworm
H. major (O. Schmidt, 1930) Franklin, 1940 (syn. *H. avenae* Filipjev, 1934)	Oat-root eelworm, cereal-root eelworm
H. göttingiana Liebscher, 1892	Pea-root eelworm

For convenience all of the above will be referred to below as ‘species’.

Objectives

The host-range tests were started as an essential corollary to hatching experiments, the primary objective being to learn more of the status of plants causing unusual hatching responses. As more material, both eelworm and plant, became available, the scope of the work was gradually expanded until it became quite extensive, involving in all several thousand plant-eelworm tests.

SOURCES OF MATERIAL AND VALIDITY OF RESULTS

Seeds and plants were obtained mainly from the Royal Botanic Gardens, Kew, and from the botanic gardens of Cambridge and Oxford Universities. Other sources of plant material were the National Institute of Agricultural Botany, Cambridge; the Plant Virus Research Station, Cambridge; Messrs Sutton and Sons, Reading; Messrs Reckitt and Colman, Norwich; Mr J. T. Legg, East Malling Research Station; Drs H. W. Howard and G. K. G. Campbell, of the Plant Breeding Institute, Cambridge; and Mr D. J. Harberd, School of Agriculture, Cambridge. Dr S. M. Walters of the Botany School, Cambridge, kindly undertook most of the work of plant identification. Unfortunately, many of the plants involved did not reach a sufficiently advanced stage of growth, whilst the more exotic species could not be identified owing to lack of suitable keys. Those successfully identified are indicated in the tables. Most of the tests were carried out using infested soil in the open field, in plant pots, or in test-tubes. Plants on which well-developed cysts were found were recorded as hosts, no attempt being made to re-infect the type host from these cysts. Although all the infested soils used were tested, by growing host plants of all known British species of *Heterodera* in them, and found apparently to contain a single *Heterodera* species in each case, absolute purity could not be guaranteed. In view of all the above circumstances, the results given in the following tables cannot be regarded as more than provisional host-range lists, pending more thorough testing, involving the use of fully identified plant and eelworm material.

The writer first used test-tubes in host-range experiments in the spring of 1951, the method being evolved independently of, and differing somewhat from, that of Oostenbrink (1952). Drainage holes were made in the bottom of the tubes, which were filled with infested soil, or with steam-sterilized soil to which were added cysts recovered from infested soil. Consequently, the results from tube tests were no more reliable than those from pot or field tests, the only advantages being that much less plant and eelworm material per test was involved, and that cysts forming on the roots could be observed through the glass without disturbing the plant.

RESULTS

The results are presented in a series of tables. Many of the tests giving negative results are not recorded, the tables being condensed by giving only the positive and the more interesting of the negative results. A key to the notation used is given below:

*=Identity of plant checked and found correct for genus.
**=Found correct for genus and species.
+=Cysts found on the roots.
−=No cysts found on the roots.
?=Cysts very small, or attachment to root doubtful.
F=Plant failed to develop adequate root system.
B, CB, CL, G=Beet, cabbage, clover and *Galeopsis* eelworms, respectively.

TABLE I. *Comparative host ranges of beet and cabbage-root eelworms—provisional list*

		Result	
Latin name	Common name	B.	CB.
CRUCIFERAE			
Aethionema grandiflora		–	–
*A. pulchellum**		–	–
*A. stylosum**		–	–
Alliaria officinalis Bieb.**	Garlic mustard	+	.
Alyssum alpestre		–	+
*A. argenteum**		+	+
A. borzaeanum Nyar.		+	.
*A. montanum**		+	+
*A. spinosum**		+	–
Alyssum sp.*		+	+
*Arabis bellidifolia**		+	+
A. caucasia Willd.**	Garden arabis	+	+
A. muralis Bertol (syn. *A. rosea* DC.		+	–
A. verna R.Br.*		+	–
*Barbarea longirostris**		+	+
B. vulgaris R.Br.**	Yellow rocket	+	+
Berteroa incana (L.) DC.**		+	–
Biscutella auriculata L.**		+	–
*B. depressa**		–	–
B. laevigata L.**		+	+
Brassica campestris L.**	Wild turnip	+	+
B. juncea (L.) Czern. & Coss.**	Trowse mustard	+	+
B. napus L.**	Coleseed	+	+
B. napus L.**	Swede	+	+
B. nigra (L.) Koch**	Black mustard	+	+
B. oleracea L.**	Broccoli	+	+
B. oleracea L.**	Brussels sprout	+	+
B. oleracea L.**	Cabbage	+	+
B. oleracea L.**	Cauliflower	+	+
B. oleracea L.**	Kale	+	+
B. rapa L.**	Turnip	+	+
Camelina sativa (L.) Crantz.**	Gold of pleasure	+	–
Capsella bursa-pastoris (L.) Medic.**	Shepherd's purse	+	+
*Cardamine pratensis L.***	Cuckoo flower	+	+
*Cheiranthus annuus**		+	+
Cochlearia glastifolia L.**		+	+
C. officinalis L.	Scurvy-grass	+	?
Conringia orientalis (L.) Dum.**	Hare's-ear cabbage	+	–
Coronopus squamatus (Forsk.) Asch.**	Swinecress	–	+
Descurainia sophia (L.) Prantl**	Flixweed	+	+
Diplotaxis erucoides (L.) DC.**	White wall rocket	+	+
D. muralis (L.) DC.	Wall rocket	–	–
D. tenuifolia (L.) DC.	Perennial wall rocket	–	–
*Erysimum allionii**	Siberian wallflower	+	+
E. cheiranthoides L.**	Treacle mustard	+	+
E. heiraciifolium L.**		+	+

TABLE I (*cont.*)

Latin name	Common name	Result	
		B.	CB.
CRUCIFERAE (*cont.*)			
Hesperis matronalis L.**	Dame's violet	–	–
Ibris amara L.**	Wild candytuft	.	–
I. coronaria Hort.*		+	+
I. umbellata L.**	Candytuft	+	+
Isatis tinctoria L.**	Woad	+	+
Lepidium graminifolium L.*		–	–
L. latifolium L.*	Broad-leaved pepperwort	–	?
L. sativum L.**	Garden cress	+	+
Lobularia maritima (L.) Desv.	Sweet alison	–	–
Lunaria annua L.**	Honesty	+	+
L. rediviva L.*		+	+
Malcolmia africana (L.) R.Br.**		?	?
M. littorea (L.) DC.		–	–
M. maritima (L.) R.Br.**	Virginian stock	+	–
Matthiola bicornis (Sibth. & Sm.) DC.**	Night-scented stock	–	–
M. incana (L.) R.Br.**	Stock	–	–
M. sinuata (L.) R.Br. var. *glabra**	Sea stock	–	–
Moricandia arvensis (L.) DC.**		–	+
M. sonchifolia		+	+
Myagrum perfoliatum L.**		+	+
Nasturtium officinale R.Br.**	Watercress	+	+
N. microphyllum (Boenn.) Rchb.**	One-rowed water-cress	+	+
Peltaria alliacea Jacq.		+	–
Raphanus maritimus Sm.	Sea radish	+	+
Rapistrum rugosum (L.) All.**		+	+
Rorippa amphibia (L.) Besser**	Great yellow-cress	+	+
R. islandica (Oeder) Borbas**	Marsh yellow-cress	+	+
R. sylvestris (L.) Besser**	Creeping yellow-cress	–	–
Sinapis alba L.**	White mustard	+	+
S. arvensis L.**	Charlock	+	+
Sisymbrium austriacum Jacq.**		+	+
S. irio L.**	London rocket	+	+
*S. orientale L.***	Eastern rocket	+	+
Thlaspi arvense L.**	Field penny-cress	+	+
LABIATAE			
Galeopsis pyrenaica Bartl.**		+	–
G. tetrahit agg.**	Common hempnettle	+	–
Lamium album L.*	White dead-nettle	–	+
L. molle		–	–
L. purpureum L.**	Red dead-nettle	–	+
Stachys annua L.**	Annual woundwort	–	+
S. officinalis (L.) Trev.	Betony	–	–
S. sylvatica L.	Hedge woundwort	–	–

TABLE 1 (*cont.*)

Latin name	Common name	Result B.	Result CB.
PAPAVERACEAE			
Eschscholtzia californica Cham.**	Californian poppy	–	–
Papaver rhoeas L.**	Field poppy	–	–
RESEDACEAE			
Reseda alba L.**	Upright mignonette	–	–
R. lutea L.**	Wild mignonette	–	–

TABLE 2. *Comparative host ranges of beet, clover and* Galeopsis *eelworms—provisional list*

Latin name	Common name	Result B.	Result CL.	Result G.
AMARANTHACEAE				
Amaranthus caudatus L.**	Love-lies-bleeding	+	?	.
A. retroflexus L.**		–	–	.
BORAGINACEAE				
*Cynoglossum amabile**		–	–	–
*Myosotis alpestris***		–	–	–
CARYOPHYLLACEAE				
Agrostemma githago L.**	Corn cockle	–	+	.
Cerastium arvense L.*	Field mouse-ear chickweed	.	+	–
*C. perfoliatum**		–	+	+
Dianthus barbatus L.**	Sweet william	–	+	–
D. deltoides L.*	Maiden pink	+	+	.
Gypsophila acutifolia Fisch*		+	+	.
G. elegans Bieb.**		+	+	+
Lychnis coronaria (L.)**		–	+	.
L. flos-cuculi L.**	Ragged robin	–	+	–
Melandrium album (Mill.) Garcke**	White campion	.	?	–
M. rubrum (Weig.) Garcke**	Red campion	–	+	+
*Saponaria cerastoides**		–	+	–
S. ocymoides L.**		+	+	–
S. officinalis L.**	Soapwort	+	+	+
Scleranthus annuus L.**	Annual knawel	–	+	.
*Silene antirrhina***		–	+	.
S. armeria L.**	Sweet william catchfly	+	.	.
S. maritima With.**	Sea campion	+	+	–
S. nutans L.*	Nottingham catchfly	+	+	.
S. pendula L.**		–	.	.
S. quadrifida L.		+	.	.
S. saxifraga L.*		+	.	.
Spergula arvensis L.**	Corn spurrey	–	+	.
Stellaria media (L.) Vill.**	Chickweed	+	+	+
Vaccaria pyramidata Med.**		+	+	+

TABLE 2 (*cont.*)

Latin name	Common name	Result		
		B.	CL.	G.
CHENOPODIACEAE				
*Beta patellaris**		–	.	.
*B. procumbens**		–	.	.
B. trigyna Waldst. & Kit.**		+	.	.
B. vulgaris L. subsp. *maritima***	Wild beet	+	.	.
B. vulgaris L. subsp. *vulgaris***	Mangold	+	–	–
B. vulgaris L. subsp. *vulgaris***	Red beet	+	–	–
B. vulgaris L. subsp. *vulgaris***	Sugar beet	+	–	–
*B. webbiana**		–	.	.
Chenopodium album L.**	Fat hen	+	.	.
*C. ambrosoides**		–	.	.
*C. amaranticolor**		+	.	.
C. botrys L.**		–	.	.
C. ficifolium Sm.**	Fig-leaved goosefoot	+	.	.
C. polyspermum L.**	Many-seeded goosefoot	+	.	.
C. quinoa Willd.*		–	.	.
Hablitzia tamnoides		+	.	.
FICOIDACEAE				
*Tetragonia expansa***		–	?	.
LABIATAE				
Galeopsis dubia Leers**	Downy hemp-nettle	–	–	–
G. pyrenaica Bartl.**		+	+	+
G. speciosa Mill.*	Large-flowered hemp-nettle	+	.	+
G. tetrahit agg.**	Common hemp-nettle	+	–	+
Lamium album L.*	White dead-nettle	–	–	–
L. molle		–	+	.
L. purpureum L.**	Red dead-nettle	–	–	+
Leonurus cardiaca L.*	Motherwort	.	.	+
*L. sibiricus**		–	?	+
Stachys annua L.**	Annual woundwort	–	–	–
S. officinalis (L.) Trev.	Betony	–	–	–
S. sylvatica L.	Hedge woundwort	–	–	–
LEGUMINOSAE				
Anthyllis vulneraria L.**	Kidney vetch	–	–	.
*Glycine soja***	Soya bean	–	+	–
Lens sp.*	Lentil	–	+	?
Lotus corniculatus L.**	Birdsfoot trefoil	–	–	.
L. tetragonolobus L.**		–	–	.
*Lupinus albo-coccineus**		–	+	–
Medicago ciliaris Willd.**		–	?	.
M. hispida Gaertn.**	Hairy medick	–	F.	.
M. sativa L.**	Lucerne, alfalfa	–	.	–
Melilotus officinalis (L.) Lam.**	Common melilot	–	+	.
Onobrychis viciifolia Scop.**	Sainfoin	–	–	.

TABLE 2 (*cont.*)

Latin name	Common name	Result		
		B.	CL.	G.
Ornithopus compressus L.**		–	.	.
O. sativus Brot.**		–	–	.
Phaseolus multiflorus Willd.**	Runner bean	–	–	.
P. vulgaris L.**	Dwarf bean	–	–	.
Pisum sativum L.**	Garden pea	–	–	–
Trigonella caerulea (L.) Ser.**		–	–	–
*Trifolium alexandrinum**		.	–	–
T. dubium Sibth.**	Lesser yellow trefoil	.	+	–
T. fragiferum L.*	Strawberry clover	.	–	+
T. glomeratum L.*	Clustered clover	.	–	+
T. hybridum L.**	Alsike clover	.	+	–
T. incarnatum L.**	Crimson clover	.	–	–
T. medium Huds.*	Zigzag clover	.	–	–
T. ochroleucon Huds.*	Sulphur clover	.	–	–
*T. pannonicum**		.	+	+
T. pratense L.**	Red clover	–	+	+
T. repens L.**	White clover	–	+	+
*T. rubens**		.	–	–
PHYTOLACCACEAE				
Phytolacca acinosa		+	.	.
POLYGONACEAE				
Emex spinosa Campd.**		–	–	.
Fagopyrum esculentum Moench.**	Buckwheat	–	–	.
Polygonum aviculare agg.**	Knotgrass	–	–	.
P. convolvulus L.**	Black bindweed	–	–	.
P. lapathifolium L.**	Pale persicaria	.	–	.
P. persicaria L.**	Persicaria	+	–	.
Rheum rhaponticum L.*	Rhubarb	.	+	+
Rumex alpinus L.*	Monk's rhubarb	+	+	+
*R. confertus**		+	+	+
R. conglomeratus Murr.*	Sharp dock	.	+	+
R. hydrolapathum Huds.**	Great water dock	+	+	+
R. palustris Sm.*	Marsh dock	+	+	+
R. sanguineus L.**	Red-veined dock	+	+	+
R. scutatus L.**	Sorrel	.	–	–
SCROPHULARIACEAE				
Antirrhinum majus L.**	Snapdragon	–	–	–
Chaenorrhinum minus (L.) Lange** (syn. *Linaria minor* (L.) Desf.)	Small toadflax	+	.	–
Veronica persica Poir.**	Buxbaum's speedwell	–	+	+
V. spicata L.	Spiked speedwell	–	–	+
TROPAEOLACEAE				
*Tropaeolum peregrinum**		+	.	.

TABLE 3. *Host range of potato-root eelworm—provisional list*

Latin name	Common name	Result
SCROPHULARIACEAE		
*Antirrhinum asarina**		−
A. majus L.**	Snapdragon	−
A. orontium L.**	Weasel's snout	−
SOLANACEAE		
Atropa belladonna L.**	Deadly nightshade	−
Browallia roezlii		−
Datura stramonium L.**	Thorn-apple	−
*Hyoscyamus canariensis**		−
H. niger L.**	Henbane	−
Lycopersicum esculentum Mill.**	Tomato	+
*L. esculentum aureum***		+
*L. pyriforme**		+
*L. racemigerum**		+
Nicandra physaloides		−
*Nicotiana acuminata**		−
*N. affinis**		−
*N. glauca**		−
N. paniculata L.*		−
*N. rustica**		−
*N. sylvestris**		−
*N. tabacum***	Tobacco	−
Physalis angulata		−
Schizanthus pinnatus Ruiz. & Pav.**		−
*Solanum aviculare**		+
*S. capsicastrum***		−
*S. citrullifolium**		−
S. dulcamara L.**	Woody nightshade	+
S. miniatum Bernh.*		+
*S. muricatum**		+
S. nigrum L.**	Black nightshade	−
*S. nodiflorum**		−
*S. rostratum**		−
*S. tomentosum**		+
*S. villosum**		+

TABLE 4. *Host range of carrot-root eelworm—provisional list*

Latin name	Common name	Result
UMBELLIFERAE		
Daucus carotae L. subsp. *sativa***	Cultivated carrot	+
D. pulcherrimus		+
Apium dulce Mill.**	Celery	−
Carum carvi L.**	Caraway	−
Coriandrum sativum L.**	Coriander	−
Ferula glauca		−
F. orientalis		—
Heracleum persicum Desf.*		−
Levisticum officinale		−
Myrrhis odorata (L.) Scop.**	Sweet cicely	−
Oenanthe crocata L.**	Hemlock water dropwort	−
Pastinaca sativa L.**	Parsnip	−
Petroselinum crispum (Mill.) Airy Shaw**	Parsley	−
P. segetum (L.) Koch	Corn caraway	−
*Sium sisarum**		−
Scandix pecten-veneris L.**	Shepherd's needle	−

TABLE 5. *Host range of hop-root eelworm—provisional list*

Latin name	Common name	Result
CANNABINACEAE		
Cannabis sativa L.**	Hemp	+
Humulus lupulus L.**	Hop	+
URTICACEAE		
Helxine solierolii Reg.**	Mind-your-own-business	–
Parietaria diffusa Mert. & Koch**	Pellitory-of-the-wall	–
*Urtica cannabina**		–
U. dioica L.**	Stinging nettle	+
*U. membranacea**		–
U. pilulifera L.*	Roman nettle	–
U. urens L.**	Small nettle	+

TABLE 6. *Host range of oat-root eelworm—provisional list*

Latin name	Common name	Result
GRAMINEAE		
Aegilops ovata		–
A. ventricosa		–
Agropyron caninum (L.) Beauv.*	Bearded couch-grass	–
*A. desertorum**		–
A. pungens (Pers.) Roem. & Schult.	Sea couch-grass	–
Agrostis gigantea Roth**	Common bent	?
A. stolonifera L.**	Fiorin, Creeping bent	+
A. tenuis Sibth.**	Browntop	–
Alopecurus pratensis	Meadow foxtail	F.
Ammophila arenaria (L.) Link	Marram grass	F.
Apera spica-venti (L.) Beauv.**	Silky Apera	–
Arrhenatherum elatius (L.) J. & C. Presl.**	Tall oat-grass	+
*Avena abyssinica**		+
A. sativa L.**	Cultivated oat	+
A. sterilis L.*	Animated oat, Fly oat	+
A. strigosa Schreb.**	Bristle-pointed oat	+
Brachypodium ponticum		+
B. sylvaticum (Huds.) Beauv.**	Slender false-brome	+
Briza minor L.**		–
Bromus arvensis L.**		+
B. sterilis L.**	Barren brome	+
Cynodon dactylon (L.) Pers.	Bermuda-grass	–
Festuca heterophylla Lam.*		+
F. pratensis Huds.**	Meadow fescue	+
F. rubra L.*	Creeping fescue	+
F. juncifolia St Am.*		+
*Gaudinia fragilis**		–
Gastridium ventricosum (Gouan) Schinz & Thell.	Nitgrass	–
Hordeum jubatum		+
H. marinum Huds.**	Squirrel-tail grass	+
*H. spontaneum**		+
H. vulgare L.**	Barley	+

TABLE 6 (*cont.*)

Latin name	Common name	Result
GRAMINEAE (*cont.*)		
Koeleria gracilis Pers.	Crested hair-grass	+
K. phleoides		+
Lolium multiflorum Lam.**	Italian ryegrass	?
L. perenne L.**	Perennial ryegrass	+
L. temulentum L.**	Darnel	+
Phalaris minor Retz.**		+
P. brachystachys Lk.**		–
Phleum pratense L.**	Timothy	–
Poa glauca Vahl		–
P. nemoralis L.**	Wood poa	+
P. palustris L.		–
P. trivialis L.**	Rough-stalked meadow-grass	+
Secale cereale L.**	Rye	+
Setaria glauca (L.) Beauv**		–
S. italica (L.) P.B.**		–
S. viridis (L.) Beauv.**	Green bristle-grass	–
Sorghum vulgare		+
Triticum aegilopoides		+
T. aestivum L.**	Wheat	+
*T. dicoccum Schueb.**	Emmer	+
T. monococcum L.**	One-grained wheat	+
T. polonicum L.**	Polish wheat	+
T. spelta L.**	Spelt	+
Vulpia ambigua (Le Gall) A. G. More		–
V. bromoides (L.) S. F. Gray	Barren fescue	–
*V. geniculata***		+
V. myuros (L.) C. C. Gmel.	Rat's tail fescue	?
Zea mays L.**	Maize	–

TABLE 7. *Host range of pea-root eelworm—provisional list*

Latin name	Common name	Result
LEGUMINOSAE		
Lathyrus aphaca L.**	Yellow vetchling	–
*L. clymenum**		?
*L. heterophylla**		+
L. hirsuta L.**	Hairy vetchling	+
L. latifolius L.**	Everlasting pea	–
L. montanus (L.) Bernh.*	Tuberous pea	–
L. niger (L.) Bernh.*	Black pea	+
L. nissolia L.**	Grass vetchling	+
L. ochrus Tourn.*		+
L. odoratus L.**	Sweet pea	–
L. palustris L.*	Marsh pea	+
L. pratensis L.**	Meadow vetchling	–
L. sativus L.**		+
*L. tingitanus**		–
Lens esculenta Moench.**	Lentil	+
Ornithopus compressus L.**		?
O. sativus Brot.**		?
Pisum jomardi Schrank*		+
P. sativum L.**	Garden pea, field pea	+

TABLE 7 (*cont.*)

Latin name	Common name	Result
Vicia angustifolia (L.) Reichard*	Narrow-leaved vetch	?
*V. atropurpurea**		+
*V. calcarata**		+
V. cracca L.**	Tufted vetch	+
V. disperma DC.**	Two-seeded vetch	+
V. faba L.**		+
*V. fulgens**		+
V. lathyroides L.*	Spring vetch	+
V. lutea L.**	Yellow vetch	+
V. orobus DC.*	Bitter vetch	+
V. sativa L.**	Common vetch	+
V. sepium L.**	Bush vetch	+

DISCUSSION

As explained above, there are several reasons for regarding the foregoing tables as constituting merely provisional lists of host plants. Nevertheless, certain general conclusions can be drawn, conclusions which will be more valid if the lists of plants in the tables are considered on a generic, rather than a specific, basis.

Beet and cabbage eelworms (Table 1). Until 1952, the writer believed that cabbage eelworm parasitized only crucifers. In the summer of that year, Miss Lewis, of the N.A.A.S. Cardiff, informed the writer (private communication) that she had found cysts on *Lamium purpureum*. She believed that these were cysts of cabbage eelworm. Subsequently, the writer found cysts on the roots of this plant, and of *L. album* and *Stachys annua*, when grown in cabbage eelworm soil. Apparently none of these three was a host of beet eelworm. The writer does not know of any other hosts, outside the Cruciferae, of cabbage eelworm. Within the Cruciferae, nearly all genera tested were found to contain hosts of beet and cabbage eelworms. Exceptions were *Aethionema*, *Hesperis* and *Matthiola*, on which genera cysts of neither eelworm were found. *Coronopus* was unique in being a host of cabbage eelworm but not of beet eelworm, since in general crucifers were attacked more readily by the latter.

Beet, clover and Galeopsis *eelworms* (Table 2). The plant groups found to contain good hosts of all three eelworms were the Caryophyllaceae, Labiatae and Polygonaceae (*Rumex* spp.). Thus the Labiatae contained hosts of four eelworms, being the only group found by the writer to do so. However, hosts of four species are contained in the Leguminosae; although the writer did not find cysts of beet eelworm on any legumes, other workers have been more successful, notably Raski (1952). Other interesting groups in this table are the Tropaeolaceae, Phytolaccaceae and Scrophulariaceae (hosts of beet eelworm). In general, there was good agreement between the host ranges of clover and *Galeopsis* eelworms, although no cysts of clover eelworm were found on *G. tetrahit*.

Potato eelworm (Table 3). Although a number of hosts was found amongst

Lycopersicum and *Solanum* species, the exact botanical status of most of the infected plants could not be determined, since they were mainly non-European and difficult to identify. The most interesting plant in the group is *Solanum miniatum*, a plant very closely related to *S. nigrum*. The identity of this plant (supplied by the Royal Botanic Gardens, Kew, as *S. nigrum* subsp. *miniatum*) is almost certainly correct. According to Clapham, Tutin & Warburg (1952), it occurs as a casual in southern England.

Three species of *Antirrhinum* (Scrophulariaceae) were tested thoroughly and repeatedly, in soil heavily infested with potato root eelworm, but no cysts were found on the roots, although Franklin (1951) observed round, golden-yellow cysts on the roots of *A. majus*, recording them as probably *Heterodera rostochiensis*. More recently it has been shown that potato eelworm is not the only species of *Heterodera* with such cysts (Lownsbery, 1952), consequently the cysts observed by Franklin may have belonged to an undescribed species, native or introduced.

Carrot eelworm (Table 4). Of a great many Umbelliferae and other plants tested, only the two 'species' of *Daucus* mentioned in the table were found infected. The exact botanical status of the plant '*D. pulcherrimus*' is not known, although the generic name appears to be correct. Thus the known host range of this *Heterodera* species consists of one (at most, two) species of *Daucus*, there being no definite additions since the eelworm was first described (Jones, 1950*b*).

Hop eelworm (Table 5). The only definite addition to the list of hosts, as given by Jones (1950*a*), is hemp, *Cannabis sativa*. As Filipjev & Schuurmans Stekhoven (1941) suggested, the *Heterodera*, described as *H. schachtii* attacking hemp in South Africa, may be *H. humuli*. The host range appears to be confined to the two genera of the Cannabinaceae, *Cannabis* and *Humulus*, and a single genus of the Urticaceae, namely *Urtica*.

Oat eelworm (Table 6). *Agrostis*, *Arrhenatherum*, *Avena*, *Brachypodium*, *Bromus*, *Festuca*, *Hordeum*, *Lolium*, *Phalaris*, *Poa*, *Secale*, *Triticum* and *Vulpia* are definitely host-genera, whilst *Koeleria* and *Sorghum* probably are. Of the fifteen tribes of the Gramineae (Clapham *et al.* 1952) representatives of eight were tested, hosts being found in the six numbered consecutively 5–10, but not in tribes 14 and 15. Assuming that the tribes are numbered according to botanical relationships, numbers 5–10 form a related group within the Gramineae; but it must be admitted that few plants outside this group have been tested. Within each of the six host-tribes, host and non-host genera appear to be distributed more or less at random.

Pea eelworm (Table 7). Bonnier (1925) divided the British Leguminosae into two tribes:

(1) Loteae. Leaves terminated by a leaflet.

(2) Vicieae. Leaves terminated by a tendril or a short point.

Although this simple division into two tribes is now regarded as archaic, the Loteae having been subdivided to form several tribes, the Vicieae still form a well-defined tribe consisting of the three common genera *Lathyrus*, *Pisum* and *Vicia*, and the non-British genus *Lens*. It is only amongst these four genera that the writer has found definite hosts of pea eelworm. Moreover, members of this tribe were not attacked readily by clover or *Galeopsis* eelworms. Hence it appears that hosts of pea eelworm are virtually confined to the tribe Vicieae, whereas preferred legume-hosts of the other two species are only to be found in the Loteae. Of the native host-genera of pea eelworm, *Lathyrus* seems to be the more resistant, cysts being found on seven out of fourteen species, whereas all, or all but one, of the twelve *Vicia* species carried cysts. The host-list given here agrees well with those of Jones (1950*a*) and Oostenbrink (1951).

For all eelworms tested, marked differences were noticed in the reproductive rates on the various host and non-host groups tested. The great economic importance of this quantitative aspect of host-efficiency is receiving increased recognition. If, for example, a few small, rather empty cysts of clover eelworm are found on pea plants after prolonged testing, and if clover plants can be re-infested from these cysts (Franklin, 1945), we cannot but recognize pea as a host of this eelworm. However, the growing of peas in soil infested with clover eelworm is more likely to decrease, rather than increase, the eelworm population. It is possible that continuous cropping with such an 'enemy' or 'trap' plant might lead to selective breeding of an eelworm population capable of successfully parasitizing the crop and multiplying thereon, but such monoculture is not usual in farming practice.

Consequently there is a definite need for some elaboration of the simple system of dividing all plants into two categories, host and non-host. The system below is suggested as one containing the minimum number of categories:

(*a*) Non-hosts, not invaded.
(*b*) Non-hosts, invaded.
(*c*) Inefficient hosts, i.e. those which reduce the infection level.
(*d*) Maintainers, those which just maintain the infection level.
(*e*) Efficient hosts, those which increase the level of infection.

The system could be elaborated further; for example, the non-host categories could be subdivided into those with stimulant and those with non-stimulant diffusates. and the efficiency of all plants could be indicated by a simple index f, where f is the factor of increase or decrease, in the infection level, due to the presence of the plant; or

$$f = \text{level of infection after one 'cropping' with the plant concerned} \\ /\text{level of infection after 'fallowing' for the same period};$$

this being the type of index used by Jones (*in litt.*).

Whatever the system used, due allowance must be made for such factors as soil type and fertility, plant population, vegetative period of crop, and initial infection level. A host which is 'efficient' when the level of the eelworm population is low may be 'inefficient' at a higher population level. Probably all host plants are 'maintainers' at a certain infection level. This gives us another possible index—

the level of the eelworm population at which a given host plant, grown under standardized conditions, is a 'maintainer'.

An example of apparent difference in the efficiency of two hosts was shown by the test described below. In the spring of 1953 two small field plots were established from well-mixed soil infested with *Galeopsis* eelworm. Sampling at that time showed the eelworm population level to be 0·20 cyst/g. × 30 eggs/cyst, or 6·0 eggs/g. Red clover seed was sown in one plot, the other being planted with young seedlings of *G. tetrahit* and *G. speciosa.* The plots were sampled again in October, the *Galeopsis* plants, being annuals, having died several weeks previously, although the red clover was still flourishing. The results of the autumn sampling were

Galeopsis plot: 0·48 cyst/g. × 151·5 eggs/cyst = 72·7 eggs/g.
Clover plot: 0·15 cyst/g. × 12·7 eggs/cyst = 1·9 eggs/g.

Evidently few, if any, new cysts formed in the plot in which clover had been sown, and the egg population decreased markedly, in contrast to the increased cyst and egg populations in the plot where *Galeopsis* had been planted. However, these differences may be more apparent than real, since the clover was still actively growing and much of the eelworm population of this plot may have been in the form of larvae, free in the soil or developing in the roots, and/or immature cysts not yet 'floatable'.

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The work described in this paper formed part of a dissertation produced by the writer while an Agricultural Research Council scholar at the School of Agriculture, University of Cambridge (Winslow, 1953).

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ON THE HOST RANGE AND SYSTEMATIC POSITION OF THE BACTERIA RESPONSIBLE FOR THE YELLOW SLIME DISEASES OF WHEAT (*TRITICUM VULGARE* VILL.) AND COCKSFOOT GRASS (*DACTYLIS GLOMERATA* L.)

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(With Plate 17)

Inoculations with *Corynebacterium rathayi* (E. F. Sm.) Dowson, alone or combined with the eelworm *Anguina tritici* into the leaves and shoots of cocksfoot grass (*Dactylis glomerata* L.) and wheat (*Triticum vulgare* Vill.), fail to cause infection. Inoculations made through the soil with *Corynebacterium rathayi* and *C. tritici* (Hutchinson) Burkh. plus *Anguina tritici* cause infection in wheat but not in cocksfoot grass. The symptoms produced on wheat resemble those of yellow slime disease of cocksfoot grass. Successful infection of wheat with *Corynebacterium rathayi* or *C. tritici* is dependent on the presence, in the soil surrounding the plants, of *Anguina tritici* together with one of these bacterial species, which are considered to be so closely related as to suggest that *Corynebacterium tritici* is but a geographical variety of *C. rathayi*.

INTRODUCTION

In spite of the similarity that *Corynebacterium rathayi* (the cocksfoot grass pathogen) and *C. tritici* (the wheat pathogen) show in many of their cultural characters (Burkholder, in *Bergey*, 1948) they have been maintained as separate species because of lack of proof of the pathogenicity of the former organism grown in pure cultures (Dowson & d'Oliveira, 1935; Dowson, 1949), beside being insufficiently described. As regards *C. tritici* on wheat, it is known from the work of Fahmy & Mikhail (1925), Carne (1926), Cheo (1946) and Vasudeva & Hingorani (1952) that there exists between this organism and the eelworm *Anguina tritici* an obligate etiological relationship comparable to that found by Crosse & Pitcher (1952) in the cauliflower disease of strawberry. Infection with the bacterial disease takes place in wheat seedlings grown in soils infested with both the bacteria and the eelworms. Sabet (1952, 1954) has also shown that the grains produced on partially affected spikes may carry over the disease when planted in eelworm-infested soils. Such spikes do not bear ear-cockles as the bacterial slime prevents the eelworms from reaching the ovaries.

The present investigation was undertaken mainly to examine the systematic position of the two yellow-slime-causing bacteria. Further, the pathogenicity of *Corynebacterium rathayi* was examined and the host range of both bacteria as influenced by association with the eelworm *Anguina tritici* was investigated.

MATERIALS AND METHODS

A culture of *Corynebacterium rathayi* was obtained through the courtesy of Dr W. J. Dowson of the Botany School, Cambridge. Three isolates of *C. tritici*, one of which was obtained from W. J. Dowson, were used. The organisms were kept on meat infusion agar containing 1 % glucose.

Soil inoculations were made in the following manner: earthernware pots 20 cm. diameter were three-quarters filled with soil and steamed for 4 hr. The remaining one-quarter was filled with soil thoroughly mixed with 50 g. of autoclaved chopped potato together with one of the following suspensions prepared in meat-infusion-dextrose broth: (i) a thick suspension of *C. rathayi* taken from 4-day-old cultures; (ii) a thick suspension of the eelworm *Anguina tritici* prepared from wheat ear-cockles surface-sterilized in 1 : 1000 corrosive sublimate for 1 min.; (iii) a thick suspension of (i) plus (ii); (iv) a thick suspension of the eelworms plus *Corynebacterium tritici*; and (v) a thick watery suspension of the eelworms plus the slime produced on affected wheat plants grown in soil inoculated with *C. rathayi* and the eelworm. No attempt was made to use *C. tritici* alone for inoculation as this was done in a previous investigation (Sabet, 1952) and proved to be unsuccessful. Seeds of *Triticum vulgare*, *T. pyramidale*, *T. durum*, *T. dicoccum*, *Hordeum vulgare* v. *pallidum*, *H. distichum* v. *erectum*, *Dactylis glomerata* and *Avena sativa* were surface sterilized in corrosive sublimate, washed in tap water, and kept over moistened filter-paper for 48 hr. Seeds of *Dactylis glomerata* and *Avena sativa* were further kept at about 4° C. for 3 days. Germinating seeds were planted, twenty-five in each pot, at a depth of about 6 cm.

Inoculations were also made into the leaves and between the leaf sheaths of potted plants of *Triticum vulgare* and *Dactylis glomerata*. The inocula were prepared as above and included (i) a suspension of *Corynebacterium rathayi*, (ii) a suspension of *C. rathayi* plus the eelworms, and (iii) a suspension of the slime produced on affected wheat plants grown in soil inoculated with *C. rathayi* and the eelworm. Similar inoculations with *C. tritici*, alone or plus eelworm, were not included as these were done in a previous investigation (Sabet, 1952) and proved unsuccessful. Inoculations were made with the aid of a flamed needle and the plants were kept under bell jars for 3 days.

The methods used for pure culture studies were those generally employed for this purpose (Dowson, 1949). 'Sugars' were incorporated as 1 % solutions in peptone water with bromthymol blue as indicator. The cultures were kept at 25° C.

EXPERIMENTAL RESULTS

(1) *The pathogenicity of* Corynebacterium rathayi

Some 105 inoculations in all were made between the end of October and the end of February 1953–4 into the leaves and shoots of cocksfoot grass and wheat (*Triticum* *vulgare*), of which thirty-six inoculations were made with *Corynebacterium rathayi*, thirty-five with *C. rathayi* plus the eelworms, and thirty-four with the slime produced on affected wheat plants grown in soil infested with *C. rathayi* plus eelworm (see below). None of these inoculations caused infection with the disease.

Cocksfoot grass plants (thirty-eight in all) grown in the different types of inoculated soils mentioned above were not infected. None was infected of the eighty wheat plants grown in the soil infested with *C. rathayi* alone. Of the fifty-two wheat plants grown in eelworm-infested soil, eighteen were infected with the eelworm disease. Of the forty-eight wheat plants grown in soils inoculated with the eelworms plus *C. rathayi*, twenty-one were infected with both the eelworm and the slime disease and two were infected with the eelworm disease alone. Infection with the eelworms was revealed by the presence of galls and distortions on the leaves and spikes. Infection with the bacterial disease was revealed by the presence of long slimy yellow spots on the leaves and leaf sheaths. Infected spikes bore yellow slimy spikelets and in a few instances turned into slimy masses and failed to emerge (Pl. 17, fig. 1). These spikes bore no grains or unhealthy looking ones but no ear-cockles. The symptoms differed from those produced by *C. tritici* in the production of smaller spots and less slime on the leaves and spikes. Of the twenty-seven wheat plants grown in soils inoculated with the eelworms plus the slime produced by *C. rathayi*, seven were infected with the yellow slime disease and six were infected with the eelworm alone. From the affected plants, *C. rathayi* was re-isolated and was readily distinguished from the closely resembling *C. tritici* by certain cultural characters (see below).

(2) *Host range of* Corynebacterium rathayi *and* C. tritici

A series of infection experiments was made during December 1953 and was repeated during February 1954 using the hosts mentioned above. About fifty to seventy-five plants of each host, in each series, were grown in potted soil infested with the eelworms or the eelworms plus either *C. rathayi* or *C. tritici*. The results were assessed by counting the number of plants exhibiting symptoms of either the eelworm or the bacterial disease when 35–40 days old.

The following hosts which were not infected with the eelworms were further not infected with either bacteria: *Dactylis glomerata*, *Hordeum vulgare* v. *pallidum*, *H. distichum* v. *erectum* and *Avena sativa*. The different species of wheat (Table 1) were more or less susceptible to *Corynebacterium rathayi* and *C. tritici*. Both organisms produced similar symptoms (Pl. 17, figs. 1, 2). *C. rathayi*, however, was less aggressive on wheat and produced smaller spots and less slime on the leaves The number of plants infected with both bacteria and eelworm together was markedly more than that of plants infected with the eelworm alone. This was most noticeable in *Triticum durum* which showed resistance to infection with the eelworm but not to the combination of the bacteria and the eelworm. The results of inoculations made into this host during December 1953, for example, showed that one out of fifty-two plants was infected with the eelworm alone, but nineteen out of fifty-seven were infected with *Corynebacterium rathayi* and eelworm together.

TABLE 1. *Inoculations with* Anguina tritici, Corynebacterium rathayi *and* C. tritici *into* Triticum spp. *through soil infestation*

	No. of plants grown in soil inoculated with									
	A. tritici alone		*A. tritici* + *C. rathayi*				*A. tritici* + *C. tritici*			
				Infected				Infected		
Triticum spp.	Total	Infected	Total	E	B	E+B	Total	E	B	E+B
December 1953 series:										
T. vulgare	51	19*	74	2	2	31	77	1	2	37
T. pyramidale	49	13	73	1	3	19	74	1	1	31
T. durum	52	1	57	1	2	19	73	—	—	23
T. dicoccum	51	7	49	1	2	15	50	—	—	15
February 1954 series:										
T. vulgare	53	9	73	1	1	17	75	1	1	21
T. pyramidale	51	6	71	1	—	10	53	1	—	10
T. durum	54	—	53	—	1	8	74	1	—	17
T. dicoccum	73	1	72	1	—	4	71	—	—	2

E = Symptoms of eelworm disease alone (curls and galls on the leaves).
B = Symptoms of bacterial disease alone (yellow slimy spots on the leaves).
E + B = Symptoms of both diseases together.
* Four of these plants were also infected with the bacterial disease of unknown origin.

(3) *Characters of* Corynebacterium rathayi *and* C. tritici

Both the wheat and the cocksfoot grass organisms are short rods, arranged in a V or Y pattern when examined from cultures of potato plugs, motile when examined in hanging drops from 48 hr.-old cultures of meat-infusion-dextrose broth, Gram-positive, non-sporing, non-acid fast, and may be surrounded by a non-stainable matrix when examined from 5-day-old cultures of heart dextrose agar. Growth of either organisms is slow, becoming moderate, bright yellow, glistening, opaque with hyaline edges on meat-infusion-dextrose agar, and viscid on potato plugs, turning to dirty yellow in old cultures of *C. tritici*. In meat-infusion-dextrose broth, moderate turbidity occurs especially at the bottom. No growth is observed in Uschinsky's or Fermi's solutions. Acid is produced by either organism from xylose, glucose, fructose and glycerol. Acid production is questionable from sucrose, lactose, maltose and raffinose. *C. rathayi* produces acid from mannitol while *C. tritici* does not. No acid is produced by either organism from sorbitol or salicin. Gelatin is slowly liquefied by *C. rathayi* and *C. tritici*. Nitrites are produced in traces but hydrogen sulphide, ammonia, and indol are not produced by either organism. Copious surface growth is produced in litmus milk accompanied by slow reduction of litmus and a soft curdling.

Discussion

It is evident from the results of the inoculation experiments that successful invasion of wheat by *C. rathayi* and *C. tritici* is occasioned by their transport into the plant by the eelworm *Anguina tritici*. A similar association probably exists between *Corynebacterium rathayi* and a particular type of eelworm capable of infesting cocksfoot grass. Goodey (1940) lists four species of eelworms affecting *Dactylis glomerata* namely *Anguina dipsaci*, *A. pratensis*, *A. obtusa* and an unidentified gall-producing *Anguina* sp. The same host is also infested by *A. agrostis* (Goodey, 1933). Any of these eelworms may play an important part in the etiology of the yellow slime disease of cocksfoot grass.

The results given in this paper confirm those of most of the previous investigators in indicating that the bacteria are carried into the wheat plant by the eelworm *A. tritici*. The results are not in agreement with those of Chaudhuri (1935) which indicate that infection with the bacteria may take place without an apparent need for the eelworm. The number of plants infected with the eelworms alone is appreciably smaller than that infected with the bacteria and the eelworms together under the same conditions. This is most marked when a wheat variety relatively resistant to the eelworm disease (*T. durum*) is involved.

Corynebacterium tritici and *C. rathayi* closely resemble each other in cultural characters and differ only in action on mannitol. It is suggested that *C. tritici* is a geographical or subtropical form of the temperate *C. rathayi*, and as the two are similar in other characters, the wheat bacterium should be regarded as a variety of the cocksfoot organism. Furthermore, it may be that *C. agropyri* (O'Gara) Burkh., the cause of the yellow gum disease of western wheat grass, is also identical with *C. rathayi*, not only in cultural characters (Burkholder, in *Bergey*, 1948), but also in pathogenicity which hitherto has not been confirmed.

The author is deeply indebted to Dr W. J. Dowson, of the Botany School, Cambridge, for many useful suggestions.

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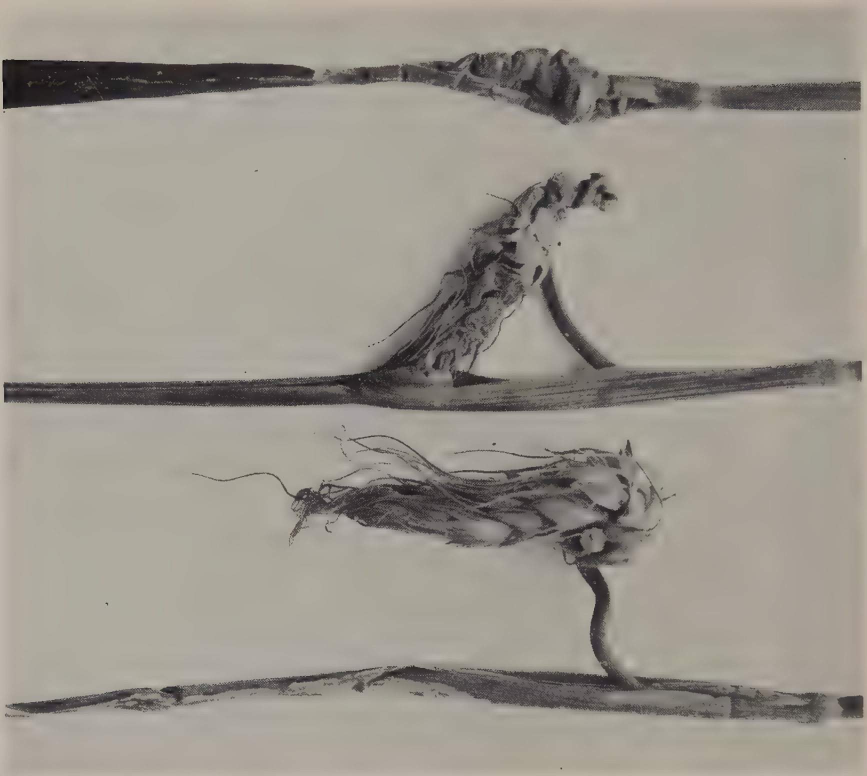
Fig. 2.

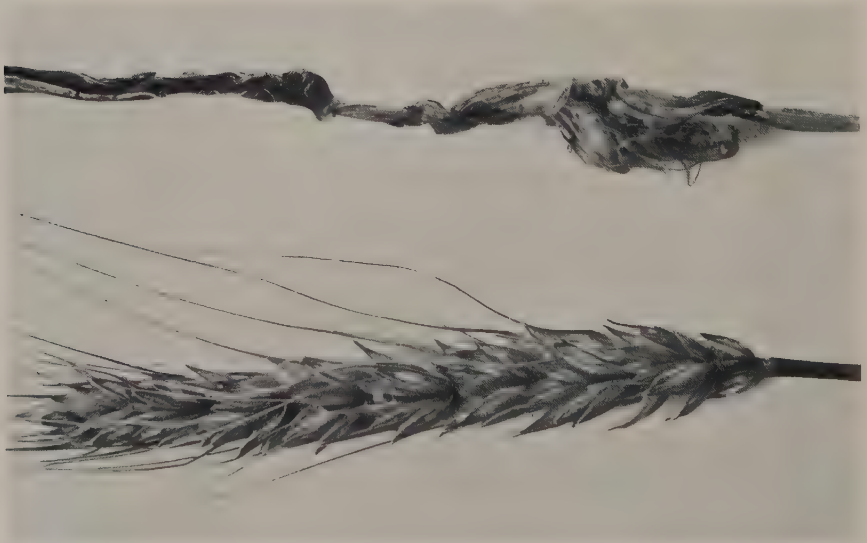
Fig. 1.

Sabet—*Yellow slime diseases of wheat and cocksfoot grass*

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EXPLANATION OF PLATE 17

Fig. 1. Wheat spikes infected with *Corynebacterium rathayi*. The spike on the left showing the common type of infection, that on the right showing exceptionally severe infection.

Fig. 2. Wheat spikes infected with *C. rathayi* v. *tritici* showing different types of infection.

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THE ANTIBIOTIC ACTION OF *BACILLUS SUBTILIS* IN RELATION TO CERTAIN PARASITIC FUNGI, WITH SPECIAL REFERENCE TO *ALTERNARIA SOLANI* (ELL. & MART.) JONES & GROUT

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The culture filtrate of a strain of *Bacillus subtilis* is shown to be antagonistic to the growth of *Rhizoctonia solani*, *R. bataticola*, *Alternaria solani*, *Botrytis cinerea*, *Fusarium udum*, and *Colletotrichum falcatum*, exhibiting specificity of action. Malformation of the germ tube of *Alternaria solani* spores resulting in 'bulb' formation is also reported. Diffusion of the antibiotic principle inside host tissues is indicated.

In the previous investigation (Vasudeva, Jain & Nema, 1952) a suitable medium for the growth of *Bacillus subtilis*, isolated by Vasudeva & Roy (1950), in relation to the production of inhibitory principle was described. The active principle was adsorbed by soil and to a less extent by kaolin but not by sand. The present investigation was undertaken with the object of studying the activities and potentialities of the culture filtrate of *B. subtilis* in relation to certain other fungi.

Material and method

The cultures of *Fusarium udum*, *Rhizoctonia bataticola*, the '*A*' isolate of *Bacillus subtilis* isolated by Vasudeva & Roy (1950) and those of *Rhizoctonia solani*, *Botrytis cinerea* and *Colletotrichum falcatum* were obtained from the Indian Type Culture Collection of fungi and that of *Alternaria solani* was isolated from infected tomato leaves. The methods of production of the inhibitory principle and testing against fungi, described by Vasudeva *et al.* 1952, were followed throughout the experimental work.

Experimental

Antagonistic activity of Bacillus subtilis *against certain plant pathogenic fungi*

The effect of the culture filtrate of *B. subtilis* was studied against six parasitic fungi on potato-dextrose-agar. The results obtained are recorded in Table 1.

The data obtained show that the culture filtrate inhibits the growth of *Rhizoctonia solani*, *R. bataticola*, *Colletotrichum falcatum*, *Botrytis cinerea*, *Alternaria solani*, and *Fusarium udum*, *Alternaria solani* and *Rhizoctonia bataticola* being most sensitive to the effect of the filtrate.

TABLE 1. *Effect of culture filtrate of* Bacillus subtilis *on certain parasitic fungi*

Fungus tested	Diameter of colonies (cm.): Control without filtrate	Diameter of colonies (cm.): With 10% filtrate	% Inhibition
Fusarium udum	7·7	2·8	63·6
Rhizoctonia solani	9·0	3·6	60·0
R. bataticola	8·9	1·3	85·4
Colletotrichum falcatum	7·9	2·6	67·1
Botrytis cinerea	8·6	4·5	47·7
Alternaria solani	8·8	1·2	86·4

Antagonistic activity of Bacillus subtilis *against* Alternaria solani

In the previous experiment it was found that the culture filtrate of *B. subtilis* inhibited the growth of mycelium of *A. solani* on potato-dextrose-agar. To examine the effect of culture filtrate on the spores of the fungus, serial dilutions of the filtrate were prepared by mixing measured quantities of sterile water with the filtrate in test-tubes, in which the spores were suspended. One drop of spore suspension from each dilution was placed on a grooved slide in a moist Petri dish and incubated at 30° C. Germination percentage was recorded at regular intervals and the results obtained are presented in Table 2.

TABLE 2. *Effect of culture filtrate on the germination of* Alternaria solani *spores*

Filtrate concentration		Incubation (hr.) 6	15	24	48	72
0% (control)	% Germination	61·6	95·0	98·0	97·5	98·6
	Germ-tube length in μ	16·0	70·0	98·5	112·0	—
1%	% Germination	19·9	37·0	44·9	78·7	79·9
	Germ-tube length in μ	12·2	38·5	85·7	87·5	—
5%	% Germination	10·2	16·1	35·5	44·0	53·8
	Germ-tube length in μ	14·0	29·7	62·0	63·0	—
10%	% Germination	Trace	7·2	6·4	8·1	9·2
	Germ-tube length in μ	—	13·3	36·7	40·0	—
25%	% Germination	Trace	Trace	Trace	5·8	5·5
	Germ-tube length in μ	—	—	—	12·1	—
50%	% Germination	Nil	Nil	Nil	Nil	Nil
	Germ-tube length in μ	—	—	—	—	—
100%	% Germination	Nil	Nil	Nil	Nil	Nil
	Germ-tube length in μ	—	—	—	—	—

Table 2 shows that there was considerable reduction in the germination percentage of spores in the culture filtrate even when diluted up to 10 times. Germ-tubes of spores treated with the antibiotic developed bulb-like structures. To find whether the inhibition produced by the culture filtrate was fungistatic or fungicidal, spores of *A. solani* were pre-treated with the culture filtrate for varying times, washed twice by repeated centrifugation in distilled water and then tested for viability. The data given in Table 3 show that after 48 hr. treatment the spores lost viability. These results indicate both the fungistatic and fungicidal nature of the antibiotic.

TABLE 3. *Germination of* Alternaria solani *spores as influenced by pre-treatment with culture filtrate of* Bacillus subtilis

Period of pre-treatment (hr.)	% germination after			
	6 hr.	15 hr.	24 hr.	48 hr.
6	40·5	72·2	91·1	90·6
15	10·6	16·5	24·3	28·4
24	Nil	Trace	Trace	Trace
48	Nil	Nil	Nil	Nil
Control	64·0	98·0	98·8	98·4

In other experiments the effects of the culture filtrate on germ tubes of *A. solani* were studied. The spores were germinated in a drop of water for 6 hr. at 30° C. on grooved slides in moist Petri dishes, and then two drops of culture filtrate from a 1 ml. pipette were placed over the germinated spores. Observations recorded at regular intervals are given below:

Treatment	Mean length of germ tubes* in μ at		
	15 hr.	24 hr.	48 hr.
Two drops of filtrate added to 1 drop water	24·8	23·1	24·5
Control (water)	66·1	105·0	118·5

* The values are means of separate sets of replicates.

It was observed that further growth of the germ tube was inhibited by the addition of the culture filtrate. Moreover, tips of the germ tubes formed bulb-like structures due to the action of the filtrate.

To test the possibility of utilizing the culture filtrate of *Bacillus subtilis* in controlling early blight of potato, inoculation experiments were conducted with detached leaves using the technique described by Wood (1951), with the following modification. Leaves were washed in tap water and surface-sterilized with 1 : 1000 $HgCl_2$ for 10–15 sec. They were then washed thoroughly in sterile water and dried between sterile blotting paper. A ring of melted sterile vaseline, approximately 10 mm. in internal diameter was painted centrally on the leaf lamina. The culture filtrate and spore suspension of *Alternaria solani* were pipetted using a 1 ml. pipette separately or in mixtures on the ringed surface. Two drops of the filtrate or suspension of spores were sufficient to cover the whole surface. The leaves were incubated in moist Petri dishes at room temperature (22–27° C.). Observations are recorded in Table 4.

There was no fungal infection when the filtrate was applied simultaneously or 8 hr. before or after inoculation with *A. solani*. With delay in the addition of the filtrate, there was a regular increase in the infectivity. Symptoms appeared in the controls on the fourth day after inoculation.

Based on the results obtained in the above experiment, further inoculations were carried out on potted potato plants. Leaves were inoculated as described earlier and the plants were incubated in humid chambers at 16–21° C.

TABLE 4. *Effect of addition of culture filtrate on infection by* Alternaria solani *on detached potato leaves*

Treatment	No. of leaves inoculated	No. of leaves infected
1 drop of filtrate and 1 drop of spore suspension added simultaneously	20	0
1 drop of filtrate + 1 drop of spore suspension added after 4 hr.	20	0
1 drop of filtrate + 1 drop of spore suspension added after 8 hr.	20	0
1 drop of filtrate + 1 drop of spore suspension added after 12 hr.	20	0
1 drop of filtrate + 1 drop of spore suspension added after 24 hr.	20	3
1 drop of filtrate + 1 drop of spore suspension added after 48 hr.	20	10
1 drop of spore suspension + 1 drop of filtrate added after 8 hr.	20	0
1 drop of spore suspension + 1 drop of filtrate added after 24 hr.	20	4
1 drop of spore suspension + 1 drop of filtrate added after 48 hr.	20	8
Control	20	18

Observations recorded in Table 5 show that the culture filtrate effectively checked infection by the fungus.

To determine whether the culture filtrate would diffuse through plant tissues, cut ends of freshly detached healthy leaves and shoots were dipped for 6 hr. in the filtrate, and later inoculated with equal amounts of inoculum. The leaves, after inoculation, were incubated in moist Petri dishes at room temperature (25–28° C.). Data presented in Table 6 show that there was less infection in treated plants than in the untreated ones, thus indicating that the antibiotic principle is capable of diffusing through the plant.

The fact that inhibition was not complete indicated the possibility that the principle was inactivated partially or wholly by the contents of the host tissue. To test this, a water extract of the leaf was prepared by grinding fresh potato leaves in a mortar using distilled water at the rate of 1 ml. per g. of tissue. The extract obtained was diluted with twice the volume of sterile water and allowed to stand for 3 hr., when the semi-clear juice was filtered and autoclaved at 10 lb. pressure for 10 min. Serial dilutions of the culture filtrate of *Bacillus subtilis* were made by

TABLE 5. *Effect of culture filtrate on controlling early blight infection of potted potato plants*

Treatment	No. of leaves inoculated	No. of leaves infected
1 drop of spore suspension and 1 drop of filtrate added simultaneously	20	0
1 drop of spore suspension + 1 drop of filtrate added after 24 hr.	20	1
1 drop of spore suspension + 1 drop of filtrate added after 48 hr.	20	6
1 drop of spore suspension + 1 drop of filtrate added after 72 hr.	20	16
1 drop of filtrate + 1 drop of spore suspension added after 4 hr.	20	3
1 drop of filtrate + 1 drop of spore suspension added after 8 hr.	20	4
1 drop of filtrate + 1 drop of spore suspension added after 12 hr.	20	6
1 drop of filtrate + 1 drop of spore suspension added after 24 hr.	20	18
1 drop of filtrate + 1 drop of spore suspension added after 48 hr.	20	19
1 drop of filtrate + 1 drop of spore suspension added after 72 hr.	20	20
Control	20	20

TABLE 6. *Activity of the culture filtrate on infection by* Alternaria solani

Treatment	No. of leaves inoculated	No. of leaves infected
Potato shoots dipped in filtrate	25	11
Control	25	19
Potato leaves dipped in filtrate	15	7
Control	15	12

mixing measured quantities of sterile leaf extract and spores of *Alternaria solani* germinated in drops of diluted extracts. The results are given in Table 7.

The results obtained in this experiment compare favourably with those given in Table 2 where the dilutions were made with sterile water in place of sterile leaf extract and show that the autoclaved leaf extracts did not have an adverse effect on the inhibitory action of the antibiotic principle produced by *Bacillus subtilis*.

DISCUSSION

The present studies show that the strain of *B. subtilis* produces an antibiotic principle which inhibits some important plant pathogenic fungi. The degree of inhibition varies in each case, indicating a certain amount of specificity of the anti-

TABLE 7. *Germination of* Alternaria solani *spores in culture filtrate of* Bacillus subtilis *as influenced by leaf extract*

Filtrate concentration		Incubation (hr.)				
		6	15	24	48	72
0%*	% germination	73·5	98·5	99·0	98·0	98·0
	Germ-tube length in μ	42·3	80·5	98·0	115·5	—
1%	% germination	64·9	86·0	88·0	87·0	86·6
	Germ-tube length in μ	35·6	78·0	97·3	99·7	—
5%	% germination	58·2	62·5	70·0	71·5	72·8
	Germ-tube length in μ	29·7	71·7	74·2	74·9	—
10%	% germination	34·8	59·0	61·4	65·2	64·4
	Germ-tube length in μ	21·3	49·0	53·7	51·2	—
25%	% germination	10·6	11·0	9·0	10·2	10·6
	Germ-tube length in μ	14·0	21·0	23·5	22·7	—
50%	% germination	—	—	Trace	Trace	Trace
	Germ-tube length in μ	—	—	—	—	—

* Control: pure leaf extract.

biotic in its antifungal action. The effect of an antifungal substance may be either fungicidal or fungistatic; in the latter case it may be accompanied by abnormal development of hyphae (Brian & Hemming, 1945; Vasudeva & Roy, 1950). The antibiotic principle produced by *B. subtilis* was found to be both fungicidal and fungistatic. Further, it induced very characteristic 'bulb' formation both on the spores and germ tubes of *Alternaria solani.*

Blanchard (1951) and Brian, Wright, Stubbs & Way (1951) have demonstrated the stability of antibiotic principles produced by organisms when introduced into host tissues, whereas Cavallito & Bailey (1944) and Timonin (1946) have reported on the inactivation of certain inhibitory principles within the host tissues. These studies have indicated that the principle from *Bacillus subtilis* is active within tissues of the potato plant. Further, results presented here indicate that it is capable of diffusion within the host tissue.

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A SURVEY OF WILD OATS (*AVENA FATUA* AND *A. LUDOVICIANA*) IN ENGLAND AND WALES IN 1951

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(With 1 Text-figure)

In 621 samples of wild oats, collected in England and Wales by N.A.A.S. and N.I.A.B. Officers, only two species (*Avena fatua* and *A. ludoviciana*) were found but both were very variable. *A. fatua* occurred in all wheat- and barley-growing areas, in both winter and spring corn and on all soil types. *A. ludoviciana* occurred (with two exceptions) only within approximately 80 miles radius of Oxford, mainly on heavy soils and chiefly in winter corn.

The amount of wild oats present appeared to depend on the frequency in the rotation of corn or other crops in which wild oats would shed seed. Deep ploughing often increased the infestation. The data did not show whether a field could be freed of wild oats by grassing down for a number of years, nor what is the maximum period of survival of buried wild oat seeds.

INTRODUCTION

Wild oats constitute a serious weed problem in cereal growing in England, and their biology is being investigated at Rothamsted to provide a basis for more effective control measures. When the work described in this paper was started, two species, *Avena fatua* and *A. ludoviciana*, were known to occur, and experiments and observations on the two species as found on Rothamsted farm (Thurston, 1951 *a*, *b*) had shown that they differed in a number of respects, the most important being the time of germination in the field. Very little was known about the distribution and relative importance of *A. fatua* and *A. ludoviciana* in England and Wales, or about the morphological and physiological variations shown by the two species in this part of their range. In order to obtain further information and to see if any other species of wild oats occurred, samples of wild oat ears or seeds were collected by officers of the National Agricultural Advisory Service and of the Seed Production Committee of the National Institute of Agricultural Botany and sent to Rothamsted for examination. The results of the survey are summarized in this paper. As an aid to identification, and in order to provide seeds of different types of wild oats grown under similar conditions for use in comparison of dormancy, some wild oat seeds from the Survey samples were grown in pots. The results of this work will be described in a later paper.

METHOD OF SAMPLING

The collectors, all of whom visited numerous farms in the course of their work, were asked to look for wild oats and to obtain specimens of the ears or seeds wherever they were seen. About twenty ears were taken from each field from points as widely distributed as possible, but a few samples from heavily infested fields contained up to 100 ears.

Information on the locality from which the samples came, the soil, the rotation, the number of ears per square yard or per acre and the duration of infestation, was asked for and usually supplied, sometimes with additional notes, for example, about departures from the usual depth of ploughing, or methods of control which had been tried.

The method of sampling produced data on the distribution of species and varieties of wild oats and on previous history of infested fields, but it furnished no comparisons between infested and uninfested fields. As the fields from which the material came were not a random sample of all possible fields, the results do not give accurate information on the level of infestation in different districts. The fact that wild oats were not collected in any district does not prove that they were completely absent, though it indicates that they were not present in sufficient quantity to constitute a serious problem.

IDENTIFICATION OF SPECIES

The true wild oats, *A. fatua* and *A. ludoviciana*, were identified by the presence of an abscission scar surrounded by hairs at the base of each spikelet. Ears or loose spikelets with these characters were then divided into *A. fatua* in which every floret in the spikelet has an abscission scar and *A. ludoviciana* with an abscission scar on the first floret of the spikelet only and a short stiff rachilla joining the 2nd and 3rd florets firmly to the first. Cultivated oats and *A. strigosa* have no abscission scars and the rachillae are more slender and easily broken than in *A. ludoviciana*. *A. strigosa* is distinguished by the two bristle-points at the end of the lemma. These diagnostic characters are shown in a key in Table 1. They can be recognized even in unripe ears.

TABLE 1. *Key for separating wild and cultivated oats on spikelet articulation*

Abscission scar surrounded by hairs at base of spikelet. Grain readily shed	All florets have abscission scar at base. Seeds of spikelet separate when ripe	=*A. fatua* (common or spring wild oat)
	Only the first floret has abscission scar. All seeds of the spikelet fall together when ripe	=*A.ludoviciana* (winter-germinating wild oat)
Neither abscission scar nor hairs at base of spikelet. Grain not readily shed	No bristle-points on lemma	=*A. sativa* (ordinary cultivated oat)
	Tip of lemma prolonged into two long hair-like points	=*A. strigosa* (bristle-pointed oat)

No new species of wild oats were found. *A. strigosa*, which resembles a wild oat but is a relic of cultivation, was collected in Northumberland, Berkshire, Buckinghamshire and Cornwall. Two out of the four samples had been recognized as *A. strigosa* by their senders, and included as being of interest for comparisons with wild oats. The Berkshire specimen occurred in oats imported from Scotland where *A. strigosa* is cultivated in certain areas; this species is also occasionally grown in dredge-corn in south-west Cornwall.*

Cultivated oats (*A. sativa* vars.), occurring as rogues in other cereals, were often mistaken for wild oats. Three samples consisted entirely of *A. sativa* and seventy-seven others contained one or more ears of cultivated oats.

Bromus sterilis had been mistaken for wild oats in two samples.

DISTRIBUTION OF *AVENA FATUA* AND *A. LUDOVICIANA*

Altogether 621 samples of wild oats were examined, 571 from the N.A.A.S. and fifty from N.I.A.B.

A. fatua was found in 97% of the samples. Table 2 and Fig. 1 show that it is widespread in England, occurring in all provinces of the N.A.A.S. but chiefly in the wheat- and barley-growing areas. The only counties reported free of wild oats were those concerned mainly with animal husbandry. *A. fatua* was also found on five farms near Newport, Mon., although Wales was believed by the N.A.A.S. to be almost free of wild oats. *A. ludoviciana* has since been found in Flintshire by Mr D. J. Griffiths of the Welsh Plant Breeding Station, Aberystwyth.

A. ludoviciana was less widespread than *A. fatua*, occurring in only eighty samples, 13% of the total number examined. It was confined to an area within approximately 80 miles radius of Oxford (Table 2 and Fig. 1). It was found in at least two-thirds of the samples from Bedfordshire, Berkshire and Buckinghamshire, in both of the samples from Oxfordshire and in the only one from Middlesex. It was present in a smaller proportion of the samples from neighbouring counties.

According to Mr C. E. Hubbard of Kew Herbarium the first records of *A. ludoviciana* in England, were in 1914 at Falmouth Docks, in 1917 at Hythe Quay, Colchester, and in 1926 at Abingdon, Berks. The latter record is particularly interesting as it is in an agricultural area very near the centre of the 1951 range of the species. This suggests that the present distribution of *A. ludoviciana* may be due to its gradual spread outwards from a centre to which it was introduced over 25 years ago. It is possible that it came in wheat imported from France during the 1914–18 war, as *A. ludoviciana* was already common in south-west France (Chevalier, 1925).

RELATION OF WILD OATS TO SOIL TYPE

The soil-type was stated for 500 samples, 435 containing only *A. fatua*, fifteen containing *A. ludoviciana* and fifty in which both species were present. Descriptions of soil-type varied from vague terms such as 'heavy soil' to precise geological nomenclature, e.g. Bembridge marl over limestone, and no doubt some of the 122 different descriptions of soil overlapped. It was possible to group the soils into broad types (Table 3), though some soils came into more than one category.

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* It is not known how the other two specimens came to be where they were found.

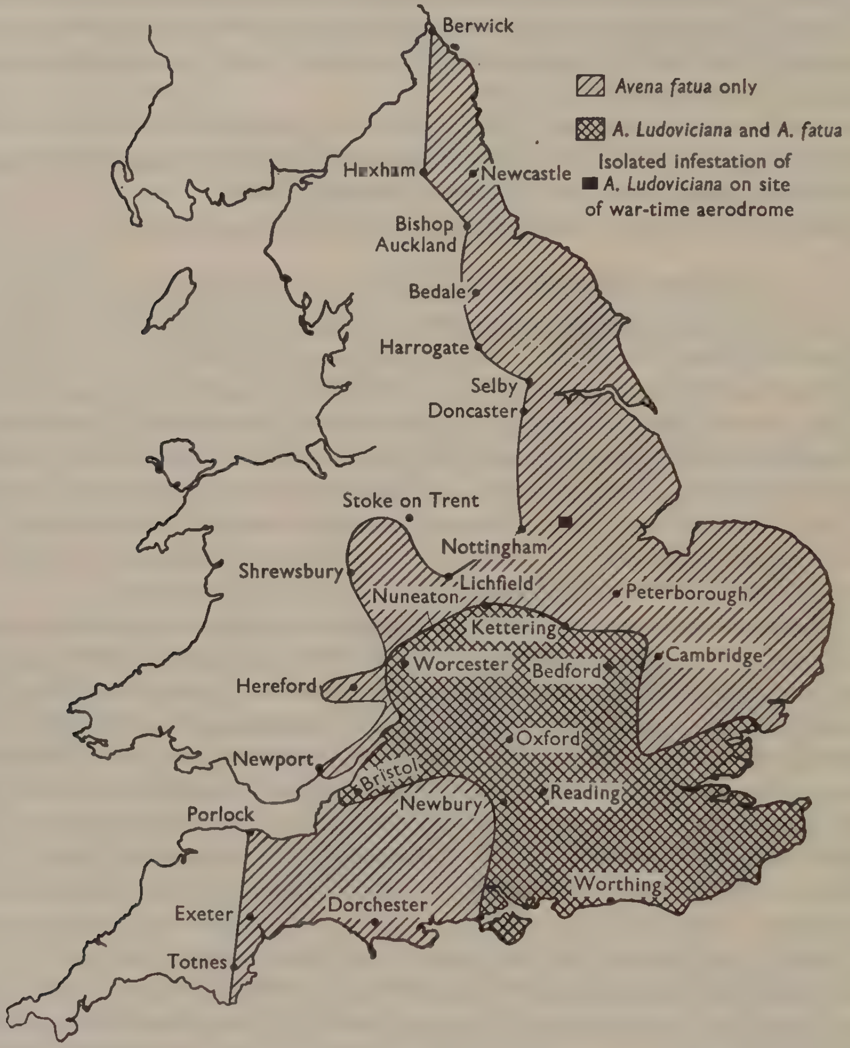
Berwick
▨ *Avena fatua* only
▩ *A. Ludoviciana* and *A. fatua*
■ Isolated infestation of *A. Ludoviciana* on site of war-time aerodrome
Hexham
Newcastle
Bishop Auckland
Bedale
Harrogate
Selby
Doncaster
Stoke on Trent
Nottingham
Shrewsbury
Lichfield
Nuneaton
Peterborough
Kettering
Worcester
Bedford
Cambridge
Hereford
Oxford
Newport
Bristol
Reading
Porlock
Newbury
Worthing
Exeter
Dorchester
Totnes

Fig. 1.

(*a*) *Avena fatua*

The soils on which *A. fatua* was found covered the whole range of physical and chemical properties of agricultural soils in England and Wales (Table 3). Acid, neutral and alkaline, high, medium and low organic content and particle sizes from clays to gravels were all represented. The pH ranged from 4·2 to 8·4. *A. fatua* was

TABLE 2. *Distribution of samples of wild oats collected in N.A.A.S. Provinces and Counties of England and Wales in* 1951

	No. of samples		
Province or county	Total	Containing *A. fatua*	Containing *A. ludoviciana*
Northern	**10**	**10**	**0**
Cumberland	0	0	0
Durham	9	9	0
Northumberland	1	1	0
Westmorland	0	0	0
Yorks and Lancs	**58**	**58**	**0**
Yorkshire	58	58	0
Lancashire	0	0	0
East Midland	**164**	**164**	**4**
Derbyshire	0	0	0
Leicestershire	35	35	0
Lincolnshire (Kesteven and Lindsey)	100	100	0
Northamptonshire	13	13	4
Nottinghamshire	14	14	0
Rutland	2	2	0
West Midland	**56**	**54**	**12**
Herefordshire	6	6	0
Shropshire	4	4	0
Staffordshire	2	2	0
Warwickshire	20	19	8
Worcestershire	24	23	4
Cheshire	0	0	0
Eastern	**148**	**143**	**14**
Bedfordshire	6	5	4
Cambridgeshire and Isle of Ely	28	24	4
Essex	35	35	5
Hertfordshire	7	7	1
Huntingdonshire and Soke of Peterborough	12	12	0
Lincolnshire (Holland)	9	9	0
Norfolk	38	38	0
Suffolk (East and West)	13	13	0
South Eastern	**129**	**117**	**45**
Berkshire	18	15	12
Buckinghamshire	24	20	19
Hampshire	23	20	4
Isle of Wight	18	18	1
Kent	12	12	1
Middlesex	1	1	1
Oxfordshire	2	1	2
Surrey	2	2	0
Sussex (East and West)	29	28	5
South Western	**51**	**51**	**5**
Cornwall	0	0	0
Devonshire	4	4	0
Dorset	18	18	0
Gloucestershire	11	11	4
Somerset	17	17	1
Wiltshire	1	1	0
Wales	**5**	**5**	**0**
Glamorgan	0	0	0
Monmouthshire	5	5	0
Other counties in Wales	No samples received, but no definite reports of absence		
Total	621	602	80

0 = wild oats looked for but not found. Many samples contained both *A. fatua* and *A. ludoviciana*

Total number of samples excludes those which contained only cultivated oats, *A. strigosa* or *Bromus sterilis*.

present on every soil-type mentioned in the Survey except Coral Rag (a sandy loam derived from limestone), but there was only one sample from this soil type so *A. fatua* cannot be assumed to be absent from it. Kimmeridge clay appeared to be unfavourable to *A. fatua* as only one of the three samples from it contained this species, and then only three ears out of forty.

TABLE 3. *Number of samples of* Avena fatua *and* Avena ludoviciana *on different types of soil.*

Soil type	No. of samples *A. fatua*	 *A. ludoviciana*
Clays	151	38
Heavy loams	50	11
Medium loams	159	9
Light loams	58	5
Sands and gravels	28	3
Calcareous	95	15
Peat and fen	24	0
Total samples	485	66

N.B. Some soils occur twice in the above grouping e.g. marls under clay and under calcareous.

In view of the general observations that *A. fatua* could thrive on all types of soil, including sand, it was surprising to hear from a farmer at Asgarby, Lincs, that wild oats were never seen on sandy soil on his farm, even where the clay soil at the other end of the same field was infested with *A. fatua*. Farmers and agricultural officers from Spalding, Lincs, and Swaffham, Norfolk, also stated that *A. fatua* was commoner on heavy land but at Tickhill near Doncaster, Yorks, on magnesian limestone, *A. fatua* was more prevalent on part of the field where the soil was lighter.

(*b*) *Avena ludoviciana*

In marked contrast to the wide distribution of *A. fatua*, *A. ludoviciana* was found mainly on heavy soils (Table 3). Of the sixty-six samples containing *A. ludoviciana* for which the soil-type was given, forty-nine were on heavy loams or clay and only seven on light soils, the remainder being on various medium soils. Fifteen were from calcareous soils but none of the soils on which *A. ludoviciana* was found was reported to be acid. There were no samples of *A. ludoviciana* from peat or fen soils. This was probably because most of the Fens lay outside the range of *A. ludoviciana* rather than because fen soil was unfavourable to the species.

However, the limited range of *A. ludoviciana* in England cannot account for its association with heavy soils. For example, Woburn Experimental Farm is on a sandy hill in Bedfordshire and is free from *A. ludoviciana*, although heavy infestations occur on the clay and brickearth of the neighbouring valleys. This is not the result of growing exclusively spring-sown cereals on the Experimental Farm, as some plots have carried winter wheat almost every year since 1877 without becoming infested with *A. ludoviciana*.

CROPS IN WHICH WILD OATS WERE FOUND

The fifty samples supplied by the National Institute of Agricultural Botany were all collected during the inspection of cereal seed crops, and emphasize the importance of wild oats as a cause of rejection of fields of pedigree seed.

The samples from the National Agricultural Advisory Service presented less biased information on the range of crops in which wild oats occurred and the frequency in each. The relation between wild oats and crop can be summarized for 536 samples. As might be expected, 86% of the samples came from cereals. Peas and field beans together accounted for a further 6·5%, roots for 2·6%, fodder crops 2·6%, flax and linseed 2·2%, and there were single samples from turnips for seed, ryegrass for seed, and coriander.

A. fatua occurred in all the crops, but *A. ludoviciana* was more restricted in range. This may have been partly due to the less frequent occurrence of that species, combined with the small number of samples from some of the crops. Sowing date also influenced the occurrence of this winter-germinating species, 24% of the winter-sown crops and only 11% of the spring-sown crops containing it. The actual date of sowing of the spring crops was rarely stated, but it seems probable that those infested with *A. ludoviciana* were sown early, before the end of the germination period of the species. The association of *A. ludoviciana* with winter wheat is probably partly correlated with its prevalence on heavy soils, since the corn on heavy land is frequently winter-sown.

RELATION OF WILD OATS TO CROP ROTATION

The crops grown in 1951 and in the two or three preceding years were known for most of the fields sampled. These crops could be divided into two classes, according to whether wild oats would tend to increase or decrease in them. Those in which wild oats would germinate readily and produce abundant seed, shedding before harvest and hence increasing the infestation in that field, are wheat, barley, oats, dredge corn, peas, beans, linseed, flax, mustard, swede seed, turnip seed and coriander. The crops in which wild oats should not multiply, and in which the reserves of seeds in the soil should be reduced, are the clean-cultivated root-crops (potatoes, sugar beet, mangolds, etc.), the grazing, hay or silage crops (ley, lucerne, sainfoin, trefoil, silage mixture) and rye. Rye is the only cereal normally ripening sufficiently in advance of the wild oats to be cut and carted before the wild oat seeds shed. It was reported from Devon that specially early-ripening oats, e.g. Richland Iowa, could be harvested there before the wild oats ripened and shed. The use of early-ripening winter cereals has also been suggested, but in most places it seems unwise to rely on cereals other than rye as cleaning crops for fields infested with wild oats. *A. ludoviciana* would always ripen some of its seeds before even the earliest harvest, and if *A. fatua* was immature when the crop was cut it would probably reach the shedding stage in the stook. Leaving the crop until it was sufficiently ripe for combine harvesting would also allow some wild oats to shed and if any of the wild oats were still green their presence would complicate the drying of the grain. All cereals except rye have therefore been treated as crops in which wild oats would be expected to increase.

The list of crops in which wild oats were found shows that neither roots nor fodder-crops can be relied upon completely to prevent seeding. The amount of wild oat seed produced in these crops depends on the management of the fields, e.g. infrequent or badly timed hoeing of roots or cutting of fodder crops may allow wild oats to go to seed. However, the infestation is unlikely to build up in these crops as quickly as in the seed crops, so in summarizing rotations they are regarded as cleaning crops.

Table 4 shows that over 90% of the fields infested with wild oats had carried at least two crops in the last 4 years in which wild oats would multiply. Indeed, 50% of the infested fields had only one cleaning crop in that time and approximately 15% had none. Unfortunately no figures are available for comparing the number of cleaning crops on fields with and without wild oats. Moreover, those fields which had carried susceptible crops most frequently also tended to have the highest number of wild oat ears per acre (Table 5). A field infested with *A. fatua* at Doddington, Cambs, was divided into halves. One half had wheat in 1949 (in which the wild oats were hand-pulled), potatoes in 1950, and wheat again in 1951. There were more wild oats in 1951 than in 1949 on this half of the field and they out-numbered the wheat ears. The other half of the field had three root crops in succession and relatively few wild oats appeared in the wheat in 1951. Insufficient use of cleaning-crops in the rotation therefore seems to be one of the causes of the abundance of wild oats in England and Wales at present. It is realized, however, that unsatisfactory rotations are sometimes due to circumstances beyond the farmer's control. The high labour demands of potatoes and root crops, the impossibility of harvesting them on land which lies wet in winter, and the need for special marketing

TABLE 4. *Relation of the number of infested fields to the number of cleaning-crops in the rotation of the past 4 years*

	Fields containing wild oats			
	A. fatua		*A. ludoviciana*	
No. of cleaning crops in the 4 years 1948–51	Total	%	Total	%
Four	1	0·2	0	0·0
Three	23	5·7	6	9·7
Two (in alternate years)	82	20·4	7	11·3
Two (in successive years)	38	9·5	5	8·1
One	207	51·5	33	53·1
None	51	12·7	11	17·8
Total	402	100·0	62	100·0

arrangements for vegetables and sugar beet, are the reasons usually given for omitting these crops from the rotation.

TABLE 5. *Relation of number of wild oat ears per acre in 1951 to number of cleaning-crops in the rotation in the past 4 years*

Approximate number of wild oat ears per acre in 1951	Fields which have had: Three cleaning crops		No cleaning crops	
	Total	%	Total	%
1 to 100	10	40	1	2
100 to 500	1	4	2	5
500 to 5000	5	20	9	20
5000 to 50,000	9	36	17	38
50,000 to 100,000	—	—	11	25
100,000 to 500,000	—	—	2	5
Over 500,000 (crop smothered)	—	—	2	5
Total	25	100	44	100

THE EFFECT OF FALLOWING INFESTED FIELDS

The rotation on fifty-one fields had included a whole year's fallow in the last 4 years, and a further five fields had had a fallow year prior to 1948. The reasons for fallowing were not always stated; evidently in some fields fallowing was an attempt at controlling wild oats but in others wild oats were not noticed until after the fallow.

Three fields were said to show a definite reduction in wild oats following a fallow year, but three others showed no change.

Taking only the twenty-three fields fallowed in 1950 and known to have had wild oats previously, we find that in 1951 two had between 10 and 20 wild oat ears per sq. yd., sixteen had from 1 to 10 and only five had less than one wild oat ear per sq. yd. Even allowing for the possibility that some of the wild oats observed were sown with the crop in 1951, these figures indicate that 1 year's fallow alone is not sufficient to reduce wild oats to negligible proportions. This is to be expected from our knowledge of dormancy of wild oat seeds and is in line with experience at Rothamsted and at Woburn (Thurston, 1951*b*). Two fields fallowed twice in the last 6 years were cleaner, having 1 ear per 3 sq. yd., and 1 ear per 5 sq. yd., in 1951.

Eight fields had been fallowed for part of a year, four in summer and the rest for longer periods, but all had infestations from 1 to 20 ears per sq. yd., in 1951. This was to be expected if a full year's fallow did not greatly reduce the wild oat population.

Although a fallow year, by itself, does not eliminate wild oats it must reduce considerably the number of dormant seeds in the soil, so long as the cultivations are sufficient to prevent any plants going to seed. The Survey does not provide any evidence on this point and cannot be taken as an argument against the use of bare fallow in a cleaning rotation, although it does emphasize the uselessness of relying on one single year's treatment, however drastic, to clear infested land of wild oats.

THE EFFECT OF GRASSING-DOWN OR GROWING LUCERNE FOR SEVERAL YEARS ON INFESTATIONS OF WILD OATS

No experiments have been done on the control of wild oats by putting an infested field down to grass. Information was therefore sought in the records of fields which had carried leys of various durations or lucerne.

(*a*) *Leys.* Although there were seventy-nine fields in the Survey records which had had leys of various lengths, only half of them were definitely stated to have had wild oats before the ley was sown. In many of these the ley had ended several years before the Survey and no information was available on the presence of wild oats in the interval between. In all the rest, the first crop after ley was a cereal and there was no proof that the seed-corn had been free of wild oats. Neither was it possible to prove that wild oats had never seeded in the hay, or that straw containing wild oats had never been fed to cattle on the field. In fact, a small sample of *A. fatua* came from the second year of a ley at Woore near Crewe, Staffs, where they had developed after the field was mown for hay, and at Long Bennington near Grantham, Lincs, small ears of *A. fatua* were found in the third year of an undergrazed ley.

The most complete case-history of a ley was from Broadwell near Rugby, Warwickshire. This field had wild oats in 1943. From 1945 to 1949 it was under ley, and in January 1950 it was ploughed up and heavily disced. Spring wheat in 1950 was free of wild oats and was sold as pedigree seed. The stubble was ploughed 4 times in the autumn of 1950 prior to sowing winter wheat, and in July 1951 this crop contained 11 ears of wild oats per sq. yd., except at one end of the field which remained free. The uneven distribution shows that the wild oats were not sown with the winter wheat.

The wild oats in the 1951 sample were 23 ears of *A. fatua* and 4 of *A. ludoviciana*. It is unlikely that pedigree spring wheat was sown late enough in 1950 to miss the spring germination of *A. fatua*, although seedlings of *A. ludoviciana* may have been destroyed in the preparation of the seed-bed. The 5-year ley therefore seems to have left the soil free of viable wild oat seeds down to normal plough-depth. Possibly the final ploughing in 1951 went deeper than usual and brought buried seeds to the surface. If so, a considerable number of these deeply buried seeds must have survived to give an infestation of 11 ears per sq. yd. when they germinated.

(*b*) *Lucerne.* A farmer at Adisham, Kent, where wild oats are very prevalent, stated that very few survive after 5 years' lucerne. No counts were available.

The evidence for the survival of buried wild oat seeds under leys or lucerne is inadequate and therefore inconclusive.

OCCURRENCE OF WILD OATS ON PLOUGHED-UP OLD GRASSLAND, RECLAIMED SCRUB AND WOODLAND

Buried wild oat seeds are sometimes thought to survive for 50–70 years or more because wild oats appear in the first crop on reclaimed land. Eight such cases were investigated in the Survey, four old grass, two scrubland, one woodland and one marshland. In three of the old grass fields the first crop was wheat and there was no proof of the freedom of the seed from wild oats. The fourth field had been ploughed in 1944 and the first crop was not stated, although it was said to have contained wild oats. This was on downland at Elsted, near Midhurst, Sussex, on the site of an Iron Age settlement, but it is most unlikely that the wild oats observed in 1944 had survived in the soil for centuries.

In contrast, nine other old grass fields, had remained free of wild oats for 2–11 years after ploughing. Six of these had their first wild oats in wheat and in another the infestation started from oat ricks in the corner of the field. The origin of the other two infestations is not known.

The time between ploughing-up and the first appearance of wild oats on old marshland in Thanet, Kent, was not stated, but the District Officer there believed that they had been introduced in straw carted out on to the marsh for bullock-feeding. The sampled field was broken up in 1948 and carried wheat for 2 years. The linseed in 1951 had one plant of *A. fatua* per 4 sq. yd.

The data on the two fields cleared from scrub was insufficient to show whether the wild oats had survived or been introduced, but the woodland near Sleaford, Lincs, was almost certainly re-infested. After being derelict for 70 years, this area was cleared in 1950 and cropped in 1951. *A. fatua* was evenly distributed over the field, the wheat was full of rogue varieties and there were many plants of cultivated oats and some of other crops. Evidently the farmer, fearing a crop failure on an untried piece of land, had been unwilling to sow expensive seed-corn. This was a false economy as the crop was reasonably good, but the wild oats introduced with it reduced what might have been 60 acres of clean land to the same level of infestation as the rest of the district.

THE EFFECT OF DEEP PLOUGHING ON FIELDS INFESTED WITH WILD OATS

Increases in wild oats following deeper ploughing were reported from fourteen localities, four in Lincolnshire, two in Cambridgeshire, two fields on the same farm in Warwickshire and one each in Yorkshire, Leicestershire, Isle of Ely, Norfolk, Kent, Hampshire and Buckinghamshire. Farmers in the Kesteven division of Lincolnshire and in the Isle of Ely stated that many fields in which a few wild oats had been present for years had become seriously infested after deep ploughing. Wild oats were also said to be increasing near Malton, Yorks, and at Southrepps, Norfolk, and the District Officers ascribed this to deeper ploughing in recent years.

The soil-type was given for thirteen of the localities where increases in wild oats following deep ploughing were reported. These were three alluvium, two lias, two boulder clay, two clay loam, one medium loam over clay, one silty loam, one loam over brash and one peat fen. Most of the fields in Kesteven and the Isle of Ely, where increases following deep ploughing were said to be general, were on fen soils.

On some of these soils the deep ploughing seems to have been unintentional, the result of the final cultivation of a series penetrating deeper than usual. On others, especially the fen soils, the deeper ploughing was deliberate; the texture of fen soil can be improved by bringing a little of the clay subsoil to the surface and deep ploughing has become a common practice on such soils. The resulting increase in wild oats is probably due to a few seeds having fallen down the cracks in the soil each summer and formed a reservoir of viable seeds, possibly on top of the subsoil, sufficiently deeply buried for them to lie dormant until brought nearer to the surface by the first deep ploughing. This does not agree with the results of pot experiments on depth of sowing of wild oats, but the relation of these pot experiments to field observations has already been discussed (Thurston, 1951*b*).

The actual depth reached by the plough was rarely mentioned. A field near Gainsborough, Lincs, was ploughed to 14 in. and a few wild oats appeared although none had been noticed previously. The first crop after deep ploughing was wheat, and it is possible that wild oats were introduced with the seed-corn, although the farmer believed that the deep ploughing was responsible for the noticeable amount of wild oats. A field near Sleaford, Lincs, provided a more definite example of increase in wild oats following deep ploughing. This field was ploughed at 10 in. in 1949 and subsequently showed a marked increase in the amount of *A. fatua* present. The first crop after deep ploughing was peas, and it is unlikely that wild oats were introduced with the seed in sufficient quantity to cause a marked increase in infestation.

Most of the increases in wild oats following deep ploughing were in fields infested with *A. fatua*, probably because that is the more widely distributed species. However, three fields contained *A. ludoviciana*; one had that species alone, in another it predominated and in the third, *A. fatua* and *A. ludoviciana* were present in approximately equal amounts.

In contrast to the numerous reports of increases in wild oats following deep cultivation, there were two fields where deep ploughing appeared to have caused a decrease and another where it had no effect, but the evidence was inconclusive in all these fields.

The opinion that deep ploughing affects the amount of wild oats present in infested fields is widespread, but it is not possible to get proof of increase or to find out how it occurs, from observations of non-experimental fields at harvest. An experimental investigation is called for.

METHODS OF SPREAD OF WILD OATS

The first year in which wild oats are noticed in a field is not always the year in which they were introduced. A few scattered plants are easily overlooked, e.g. on a farm near Rugby, Warwickshire, they were first noticed by the District Advisory Officer on an inspection for take-all. The farmer had overlooked them. Larger amounts may be mistaken for cultivated oats shed from a previous crop.

It is also possible that some wild oat seeds may remain dormant in their first year in the soil. These factors must be remembered when basing deductions about methods of introduction on reports of the first occurrence of wild oats.

The following methods of introduction were all well-authenticated. They fall into two broad groups, methods by which wild oats first come on to a clean farm, and those by which the weeds subsequently spread to other fields on the same farm, but there is no hard and fast line between them.

(1) *Methods chiefly responsible for spread from one farm to another*

(*a*) *In contaminated seed-corn.* In twenty-three of the fields sampled, wild oats were said to have been introduced in seed-corn. This was certainly true in a Sussex field sown with home-grown oat seed, where the wild oats were actually in the drill-rows. In eleven more fields there was no evidence to the contrary, although the wild oats could not be proved to have been sown with the corn. These were three fields in Yorkshire and one each in Sussex, Norfolk, Bedfordshire, Hertfordshire, Berkshire, Leicestershire, Northamptonshire and Dorset. They included four fields of wheat, four barley and three oats. All contained *A. fatua*, but in the Bedfordshire field *A. ludoviciana* was also present.

In the remaining eleven fields, introduction of wild oats in seed-corn was more doubtful. The seed-corn for eight of them was obtained from reliable sources and was unlikely to have contained wild oats. One sample was imported direct from Sweden, where wild oats are almost absent in the areas producing seed-corn. The other seven were British, either N.I.A.B. stocks or stocks for a Field Approved scheme for seed-corn. Some of the officers concerned with field inspections said that farmers were often unwilling to admit that they had spoiled clean seed by sowing it in fields infested with wild oats, and that some of the reports of first occurrence in special stocks may be untrue. A field at Basingstoke, Hants, was sown with seed-corn from Norfolk and yet the wild oats found in it were *A. ludoviciana*, which is believed to be absent from Norfolk but occurs in Hampshire. The wild oats probably came from another field in the vicinity, but there is nothing to show how they got there.

Alternative explanations of the appearance of noticeable amounts of wild oats were possible in two cases; one was next to a heavily infested field and another had been deep ploughed before the cereal was sown.

In a third field, two different varieties of oats were infested with the same single type of *A. fatua*, a relatively rare occurrence as populations usually consist of more than one variant of the species. It is probable therefore that the wild oats had been introduced earlier from a single source.

Although some reports of introduction of wild oats in seed-corn do not bear examination, there are many which do. The District Agricultural Officer for mid-Staffordshire, where wild oats are normally absent, mentioned a field which showed a few wild oats each time it was in corn. It was under ley in 1951 and no sample was obtainable. The farmer said the wild oats had been introduced in seed-corn.

The presence of a plant of *A. strigosa* in Berkshire, in Victory oats from Scottish seed, also shows how a species may be spread in seed-corn.

(*b*) *From traffic passing along neighbouring cart-tracks, roads or railways.* Infestations were said to have developed beside roads or cart-tracks in fields in Suffolk, Leicestershire and the Isle of Wight, presumably from seeds shed during carting or in mud picked up on wheels and implements in infested fields and dropped along the road. In a field at East Langton, Leics, which was divided into three sections the potatoes and wheat near a railway line were infested while oats further from the railway were free of wild oats. Weed-seeds dropped from railway trucks might come from straw packing material, from bedding for animals or from cargoes of infested grain.

(*c*) *In sacks.* Owing to the hairs on the husk, wild oat seeds easily get caught up in material such as sacking. An infestation at Stoke Charity, Hants, was believed to have originated from seeds introduced in fertilizer bags previously used for seed-corn contaminated with wild oats.

(*d*) *In straw used as packing material.* A few plants of *A. ludoviciana* were found in 1951 in a field near Grantham, 40 miles beyond the range of this species as shown by the Survey. The site was on the edge of a wartime aerodrome and had been flanked by a row of storage-huts and it is possible that *A. ludoviciana* had come in straw used for packing material. The species is evidently spreading from this centre of introduction as it was reported from an adjacent field in 1952.

(2) *Methods chiefly concerned with spread from one field to another on the same farm*

(*a*) *In home-produced and inadequately cleaned seed.* Spread of wild oats to other parts of the farm in home-grown bean seed was reported from Hereford and Lincolnshire. If there are weevil holes in peas or beans, wild oats may stick in them and cannot be removed by seed-cleaning machinery.

Introduction of *A. fatua* in flax seed was claimed at Thetford, Norfolk, but it is difficult to believe owing to the difference in size and texture of the crop and weed seeds, unless the seed had not been cleaned.

(*b*) *In corn-stacks containing the produce of infested fields, and built on clean fields.* Wild oats were abundant on the sites of old stacks in six fields, two on the same farm in Warwickshire and one each in Northamptonshire, Suffolk, Lincolnshire and Somerset. Seven other fields had localized infestations in corners or at the ends where stacks might have stood. There were of course other fields with patchy infestations which were not attributable to stacks.

(*c*) *In straw for feeding animals.* Marshland in Thanet, Kent, is believed to have become infested by this means, while it was used for pasture. Wild oats appeared as soon as it was ploughed up.

(*d*) *In farmyard manure.* The spread of wild oats over a farm in Wisbech, Cambs, was attributed to the use of farmyard manure. Viable wild oat seeds have never been found in samples of well-rotted dung from Rothamsted cattle-yards, but it is possible that with less careful handling viable seeds might get in from bedding or feed, or fall on dung-piles waiting to be spread. It was also shown (Rothamsted Annual Report, 1951, 1952) that a very small proportion of seeds of *A. ludoviciana* fed to a calf passed through the animal undamaged and germinated in the dung. Spread of wild oats in dung is therefore possible, though it is not often reported.

Control methods

Comments on the success or failure of control methods which they had tried were sent by forty-five farmers, and a District Agricultural Officer collected the opinions of seventeen farmers near Sleaford, Lincs. The methods tried fell into three groups, those depending on the type of crop or the rotation, those involving soil cultivation, and a miscellany of other methods. None of the methods were new, and none were claimed to give complete eradication of wild oats.

Growing three successive crops in which wild oats would not shed was the method most often considered successful, ten successes and no failures being reported. Hand-pulling was next with nine successes and two failures. Other treatments were less often tried or gave inconsistent results. The choice between winter or spring corn for control of *A. fatua* illustrated the difference of opinion which can arise over the value of a method. As none of the opinions on control measures were supported by counts of wild oats or by data from untreated areas, they are not described in detail here.

Discussion

The Survey results agree on many points with the results of previous observations and experiments and with opinions expressed by agricultural officers and farmers in Britain (Travers, 1950; Brenchley, 1947; Brenchley & Thurston, 1948; Thurston, 1951*a*, *b*; Dadd, 1953). The prevalence of wild oats in all wheat- and barley-growing areas, their relation to the frequency of corn-crops in the rotation, the danger of spread in seed-corn, and the occurrence of *A. fatua* on all types of soil were all expected from previous experience. So was the impossibility of cleaning an infested field in a single year, however drastic the treatment, by any method available at present.

The chief contribution of the Survey to our understanding of the wild oat problem in England and Wales was the information on the range and intensity of infestations of *A. ludoviciana*. Although this species is less widespread than *A. fatua* it is present in a sufficiently great area (approximately 20,000 sq. miles) and reaches a high enough density per acre to constitute a serious weed problem by itself, even if *A. fatua* were not also present in many fields where *A. ludoviciana* occurs. Owing to the presence of the two species together in many fields, no accurate estimate of the number of ears of *A. ludoviciana* per unit area was possible, but twenty-five of the eighty fields sampled probably had more than 1 ear of *A. ludoviciana* per sq. yd. or over 5000 per acre, and at least four fields had 10 times as much.

In theory, *A. ludoviciana* should be easier to control than *A. fatua*, as its germination period ends earlier in the spring. Late spring sowing of cereals should therefore give a crop practically free of *A. ludoviciana*. However, it is associated with heavy soil and on many infested fields early autumn sowing is necessary, the land remaining unworkable until too late for spring sowing.

Another important point raised by the Survey was the increase of wild oats which may follow deep ploughing on a field where a few wild oats have occurred for years.

Some of the observations on wild oats in England and Wales agree with those in other countries. Cates (1917) advised farmers in U.S.A. to avoid ploughing *A. fatua* seeds in deeply, as it might induce dormancy and prolong their period of survival. He also noted that *A. fatua* was less prevalent where inter-tilled crops were grown, and pointed out the necessity for preventing wild oats from going to seed in hay. He stated that seeds of *A. fatua* would survive 2 years in clay soil and 4-6 years in sandy loam. Clark (1914) gave the survival of *A. fatua* seeds in soil in Canada as over 2 years but probably less than 5, and Chepil (1946) in Saskatchewan found that very few seeds of *A. fatua* survived into the fourth year. These results agree in general with the conclusion from the Survey that in England and Wales *A. fatua* cannot be controlled by a single year's treatment, but that three clean-cultivated crops in succession will give a good measure of control.

Chevalier (1925) said that *A. fatua* was absent from many parts of north-west France and was never seen on the cold siliceous soils because if occasional individuals got in they died out. This is even more emphatic than the statement by Cates (1917) on the influence of soil type on the number of years for which buried seeds of *A. fatua* will survive, and appears to contradict the conclusion of the present Survey that the distribution of *A. fatua* in England and Wales is unaffected by soil type. However, the infestation on our heavier soils may be maintained by periodical re-infestation or by the too-frequent occurrence of cereals in the rotation.

A. ludoviciana is not recorded from Canada or the U.S.A. Chevalier (1925) stated that in France it was only found south of the River Loire, i.e. that its northern limit was farther south than that of *A. fatua*, and that it was very common in south-west France. It is rather surprising that a species characteristic of south and south-west France and North Africa, but absent from north France, should become a weed in England.

Chevalier's suggestions for control of wild oats are based on the principles of cultivation and rotation, and he mentions the importance of spread of wild oats by badly cleaned seed, by travelling threshing-tackle, and by wind. There is no evidence of the latter in England and Wales.

The use of cultivations and rotation of non-susceptible crops is also recommended for control of *A. fatua* in Canada and U.S.A. (Clark, 1914; Cates, 1917). Bibbey (1935) found a 500% increase in germination of *A. fatua* due to tillage in an experiment in Saskatoon, Canada, and he also found that in 2 successive years germination of *A. fatua* occurred after the normal sowing dates of spring wheat, barley and rye. This agrees with the prevalence of *A. fatua* in spring corn in England and Wales.

Rabaté (1913), in France, stated that deep ploughing and thorough cultivation helped to destroy wild oats in the current season but favoured their occurrence in subsequent cereal crops. This was presumably due to the deeply buried seeds remaining dormant until the next time the field was deep-ploughed, and if so it is in line with observations on deep ploughing in England and U.S.A.

Conclusion

The Survey has shown the magnitude of the wild oat problem in England and Wales and the long-term nature of the only control methods available at present. Many farmers, N.A.A.S. Officers and corn merchants are taking a keen practical interest in solving the problem along traditional lines. The Unit of Experimental Agronomy at Oxford, and firms concerned with the manufacture and application of weed-killers, are also seeking for selective chemicals for controlling wild oats. Fundamental research on the biology of wild oats is needed to provide the background for successful application of control methods. In particular, more work is needed on the causes of dormancy in wild oat seeds and on methods of overcoming it, as the survival of seeds buried in soil for a number of years is one of the main difficulties in getting rid of wild oats. A fuller understanding of the ways in which wild oats are introduced to clean fields, is also needed to give greater prevention of spread.

Over 150 officers of the N.A.A.S. and N.I.A.B. helped to collect the samples and information which made the Survey possible. The farmers who supplied information and permitted sampling of their fields also played a vital part. Mr C. E. Hubbard of Kew Herbarium supplied data on the first records of *A. ludoviciana* in England. Dr D. J. Watson, head of the Botany Department at Rothamsted, has given valuable advice and encouragement at all stages of the work. The help of all these people is gratefully acknowledged.

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TOXIC EFFECTS OF HEAVY METALS ON CROP PLANTS GROWN IN SOIL CULTURE

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A number of pot experiments were made on various crops, to study the effects on the chemical composition of the plants of treating the soil with cobalt, copper or nickel sulphates, or with the cupric or ferric salts of ethylenediamine-tetraacetic acid (EDTA).

The tops of copper sulphate-treated plants usually contained higher concentrations of copper, manganese, calcium and magnesium and lower concentrations of potassium and phosphorus, than those of control plants.

The most marked effect of cobalt on oat was to increase the concentration of Ca+Mg in the tops; nickel had a profound effect on the composition of barley tops.

Total iron concentration was reduced in oat by cobalt and copper, and in barley by copper and nickel application. Symptoms of iron deficiency appeared only in the oat plants, their incidence being correlated with the reduction in iron concentration in the tops.

The ferric salt of EDTA tended to reduce the incidence of iron deficiency symptoms produced in oat by copper sulphate, but also gave rise to distinct toxic effects. Plants given the cupric salt of EDTA contained unusually large amounts of iron.

INTRODUCTION

The pollution of land by industrial effluent or as a result of mining operations has been an economic problem in some countries for a century or more. Lead toxicity in the Mendip Hills was recorded as long ago as 1668 by Glanvil (quoted by Phillips, 1882); more recently, Wallace (unpublished results) found that zinc toxicity was responsible for the failure of crops grown in certain areas of the Mendip Hills. Wallace and Morley Davies (unpublished results) investigated crops near Wolverhampton that had been poisoned by sewage which was contaminated with a variety of metals. Spectrographic analysis of these crops showed that they contained unusually large amounts of copper, nickel and zinc (R. L. Mitchell, private communication).

Griffiths (1918), Jones (1940) and Newton (1944) have studied zinc and lead toxicities occurring in certain mining areas of West Wales. Copper toxicity has been studied in the U.S.A. by Bateman & Wells (1917) and Forbes (1917), and in Germany by Freytag (quoted by Phillips, 1882) and Haselhoff (1892); whilst zinc toxicity has been investigated in Belgium by Rossels (1924), and in Germany by Freytag (*loc. cit.*) and Storp (1883). In addition, soils sometimes occur which naturally contain unusually large amounts of toxic metals. Soils giving rise to crops of high nickel content have been described by Birrel & Wright (1945), Hunter & Vergnano (1952) and Minguzzi & Vergnano (1948). Certain peat soils in New York State are of exceptionally high zinc content (Staker, 1942; Staker & Cummings, 1941).

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The present experiments were made in order to study the ways in which toxic metals affect the mineral nutrition of the plant—a subject on which little information is available. Of especial interest is the relationship between the heavy metal content of the soil and the iron level in the plant, since various workers have shown that heavy metals may induce symptoms of iron deficiency in higher plants (Somers & Shive, 1942; Millikan, 1947*b*; Hewitt, 1948).

During 1951, oats, rye, barley, sugar beet and kale were grown in soils treated with copper and, in 1952, experiments were made to study the effects of cobalt and copper on oat, and of copper and nickel on barley. Cobalt was used because of the readiness with which it has been reported to induce symptoms of iron deficiency in crop plants (Hewitt, 1948; Nicholas, 1951), and nickel because of the interest aroused by the occurrence of nickel toxicity in the field in Aberdeenshire (Hunter & Vergnano, 1952).

Finally (1953), oats were grown in soil treated with either the cupric salt of ethylenediamine-tetraacetic acid (EDTA), or with combinations of copper sulphate and ferric-EDTA. Ferric-EDTA has been used to correct the chlorosis of fruit trees grown in unfavourable soils (Alexander & Walsh, 1952; Stewart & Leonard, 1952), and it seemed of interest to determine whether ferric-EDTA would also correct the chlorosis of plants grown in soils treated with copper sulphate. Parallel tests were made with cupric-EDTA in order to compare the availability to the plant of copper added to the soil as either the sulphate, or the complex with EDTA.

EXPERIMENTAL

Each experiment was made in the glasshouse, and the crops were grown in 6 in. bitumen-painted clay pots standing in bitumen-painted saucers. Each pot contained 1·6–1·7 kg. of soil; details of the soils used are as follows:

Experiment	Soil
1951: oat, rye, barley	3 parts sandy loam mixed with 2 parts granite chips, pH 5·9
1951: sugar-beet, kale	Derived from New Red Sandstone, pH 6·4
1952: oat	Medium loam, pH 6·8
1952: barley	Potting compost, pH 6·8
1953: oat	Potting compost, pH 6·8

The varieties of the crops grown were as follows: oat (S 84), rye (Giant), barley (Plumage Archer), sugar beet (Kleinwanzleben) and kale (Borecole).

Co, Cu and Ni were all applied to the soil as solutions of the sulphates. Throughout this paper, subscripts will be used to denote the relative rates of metal application (see the table which follows).

1951 *experiments*

Details of the experiments are summarized in the following table:

	Copper application, (m.equiv.* per pot)				
	Cu_0	Cu_1	Cu_2	Cu_3	Harvest
Exp. 1, oat	0	12·5	25	50	
	Applied 4 weeks before transplanting				4 weeks after transplanting 5 weeks after sowing
Exp. 2, rye and barley	0	5	10	20	
	Applied 6 days before transplanting				3 weeks after transplanting 4½ weeks after sowing
Exp. 3, sugar-beet and kale	0	10	20	40	
	Applied 4 days before transplanting				6 weeks after transplanting 9 weeks after sowing

* Milli-equivalents.

There were 192 pots in each experiment, arranged in four blocks each of four plots (copper levels). There were twelve pots receiving the same treatment in each plot. In Exps. 2 and 3, each plot was further divided into two subplots, giving six pots for each crop.

The seeds were germinated in a potting compost, and were usually transferred to the pots of treated soil when 4–5 in. high. When the plants were well established, each pot was given 50 ml. of a solution containing 5·6 g. K_2SO_4, 5·6 g. $MgSO_4.7H_2O$ and 22·4 g. $(NH_4)_2SO_4$ per litre.

At harvest, the plants within each plot or subplot were combined. The tops were cut off approximately ¾ in. above soil level and washed under a jet of water (Exps. 1 and 2) or in a dilute Teepol solution (Exp. 3). In each experiment, the roots were freed from as much soil as possible by washing them in Teepol solution, after which they were laid on sheets of zinc gauze and washed carefully under a jet of water.

1952 *experiments*

Oat

There were 192 pots arranged in six blocks, with four rates each of Cu (0, 12·5 25 and 50 m.equiv. per pot, applied on 10 June) and Co (0, 4, 7 and 10 m.equiv. per pot, applied on 16 June) applied in factorial combination, giving two pots per block for each of the sixteen treatments. Six oat seedlings about 4 in. high were transplanted into each pot on 13 June, 8 days after sowing.

The plants were harvested on 26 June, and the twelve plants within each block receiving the same treatment combined to give a single sample. Care was taken during the course of the experiment and subsequent sampling to avoid contamination of the foliage. The plant tops were cut off just above the most mature leaf and were not washed, but any traces of dust removed with a camel hair brush. The roots were not sampled in this and subsequent experiments because of the difficulty of removing all traces of soil from them.

Barley

The experimental design was the same as in the last experiment, except that Co was replaced by Ni, and there were seven instead of six blocks, each containing thirty-two pots. Both Cu (0, 12·5, 25 and 50 m.equiv. per pot) and Ni (0, 7·5, 15 and 30 m.equiv. per pot) were applied on 29 July, and on 5 August, six barley seedlings, 10 days from sowing and about 6 in. high were transplanted into each pot. The plants were harvested 13 days later, omitting those at Cu_3 because of the high mortality. The same sampling technique was used as in the previous experiment.

1953 *experiment with oat*

Twelve treatments were applied as follows:

(*a*) Three rates of $CuSO_4$ (0, 20 and 40 m.equiv. per pot) combined factorially with three rates of ferric-EDTA (0, 1·5 and 3 m.equiv. per pot).

(*b*) Three rates of cupric-EDTA (1·5, 3 and 4·5 m.equiv. per pot).

There were two pots per block for each treatment, and eight blocks, giving a total of 192 pots. The solutions were applied on 26 May, and on 3 July eight oat seedlings 8 days from sowing, were transplanted into each pot. The plants were harvested on 14 July, using the same sampling technique as in the 1952 experiment with oat, and their dry weights and iron contents determined. It was not possible to analyse all of the samples for copper, but samples were selected to show the effect on Cu concentration of either $CuSO_4$ in the absence of ferric-EDTA, or vice versa.

Analysis of the plant samples

Samples were dried at 100° C. to constant weight, then wet-digested with nitric and perchloric acids (Nicholas, 1948). The analytical methods used were as follows:

Calcium: precipitation as oxalate, followed by $KMnO_4$ titration.

Calcium plus magnesium: titration with ethylenediamine-tetraacetic acid (EDTA) in the presence of KCN (Diehl, Goetz & Hach, 1950). Ca and Mg were determined together in the 1952 samples because insufficient material was available for the separate determinations to be made.

Copper: extraction as the diethyldithiocarbamate complex in the presence of EDTA (Forster, 1953*e*).

Iron: Moss & Mellon's (1942) dipyridyl method.

Magnesium: titration with EDTA, after first removing interfering metals (Forster 1953*c*).

Manganese: by periodate oxidation in the 1951 samples, by the tetrabase method of Nicholas & Fisher (1951) in the 1952 samples.

Nickel: colorimetric measurement of the red Ni^{IV}-dimethylglyoxime complex, after separating the Ni from interfering metals (Forster, 1953*d*).

Potassium: a cobaltinitrite procedure.

Phosphorus: Kitson & Mellon's (1944) vanadate method.

Statistical analyses

The analytical data for K, Ca, Ca+Mg, Mg and P were always normally distributed, but with the results for Cu, Mn and Ni the deviations were frequently proportional to the means at the different treatment levels, and logarithmic transformations of the data were necessary before they could be examined statistically. Iron deficiency symptoms (oat experiment 1952) were recorded as the percentage of plants affected, and so had to be transformed to angular degrees before statistical treatment could be applied.

RESULTS

1951 *experiments*

Symptoms resembling those of Fe deficiency (see Hewitt, 1949) appeared in a number of the Cu-treated oat plants 5 days after transplanting, their incidence at harvest being 0, 6, 19 and 7% of the original population at Cu_0, Cu_1, Cu_2, and Cu_3 respectively. A few of the sugar-beet plants at Cu_3 developed Fe deficiency symptoms, but the symptoms did not appear in rye, barley or kale. Purple tints developed in the leaves and stems of rye plants given Cu. The older leaves of all crops given Cu usually became necrotic or uniformly chlorotic (as distinct from the intervenal chlorosis associated with Fe deficiency).

As progressively more Cu was added to the soil, all crops showed an abrupt increase in mortality (Table 1), and a decrease in dry weight.

TABLE 1. *The effect of copper application on mortality*

	Percentage of original population affected			
	Cu_0	Cu_1	Cu_2	Cu_3
Oat	0	1	33	83
Rye	0	0	0	51
Barley	0	0	11	83
Sugar-beet	0	0	1	58
Kale	0	0	2	57

The results given in Table 2 are typical of those obtained with all five crops. Attention was given in these experiments to the concentration and not to the total amount of each nutrient present in the plant samples, since the latter was (except for Cu) always lower in treated plants than in controls, because of the marked reduction in dry weight caused by Cu application.

TABLE 2. *The effects of copper on the dry weight and nutrient status of oat*

	Rate of Cu application				
	0	1	2	3	S.E.
Tops					
Dry weight	24·8	21·2	8·6	1·6	1·04
Fe	128	120	142	101	10·5
Cu	1·16	1·49	1·94	2·60	0·12
Mn	2·37	2·58	2·68	2·81	0·03
K_2O	7·87	5·86	4·87	4·15	0·24
CaO	0·77	0·74	0·78	0·91	0·07
MgO	0·39	0·47	0·49	0·64	0·03
P_2O_5	1·43	0·99	0·88	0·91	0·05
Roots					
Dry weight	0·83	0·81	0·57	0·20	0·03
K_2O	3·16	2·87	2·32	1·89	0·13
CaO	0·85	2·12	2·11	2·39	0·09
MgO	0·42	0·58	0·48	0·85	0·04
P_2O_5	0·88	0·53	0·51	0·53	0·03

Dry weight of tops in g./12 pots; of roots as $\log_{10}$ (1 + g. dry matter/12 pots).
Fe in μg., and Cu, Mn in $\log_{10}$ μg./g. dry matter. K_2O, CaO, MgO and P_2O_5 as % dry matter.
Each value is the mean of four observations.

The changes in nutrient status following the addition of copper to the five crops can be summarized as follows:

(*a*) Decreases in the concentrations of K and P in both tops and roots. Although phosphate concentration was usually lower in treated plants than in controls, it was actually higher at Cu_3 than at Cu_1 or Cu_2 in barley tops and roots. A similar effect had been noted in an earlier experiment with oat.

(*b*) Increases in the concentrations of Cu, Mn and Ca, although Ca concentration was increased in only the roots and not the tops, of oat and kale. Mg concentration, too, was usually higher in treated plants than in controls.

(*c*) There was no consistent effect on the concentration of Fe in the plant tops. Fe concentration was decreased in oat and increased in barley, but the effects were significant only at $P = 0\cdot05$.

It was observed that rye and barley responded in a closely similar manner to Cu application, thus both crops gave similar decreases in dry weight, and similar increases in the concentrations of Cu, Mn and Ca in the tops (Forster, 1953*a*).

1952 *experiments*

Oat

Symptoms of Fe deficiency appeared a week after transplanting in the leaves of several plants given either Co or Cu. More plants were affected during the next few days, after which the symptoms began to disappear. Their incidence at harvest is given in Tables 3 and 4, which show some of the effects observed in the experiment. No mortality occurred.

The experimental results were studied in more detail by compiling a series of 4 × 4 tables (four rates each of Co and Cu; Forster, 1953*b*), and evaluating the standard errors for the Co, Cu means and Co × Cu interactions but these are too bulky to publish in full, and only the 4 × 4 table for P_2O_5 concentration is included in this paper.

TABLE 3. *The effects of cobalt and copper on oat tops*

	Metal applied	Rate of metal application				
		0	1	2	3	S.E.
Dry weight	Cu	1·12	1·03	0·99	0·70	0·03
	Co	1·03	1·00	0·94	0·87	
Fe Deficiency symptoms	Cu	8·4	23·4	34·9	53·7	3·6
	Co	4·9	31·4	36·7	47·3	
Fe	Cu	121	108	92	80	2·3
	Co	107	97	101	95	
Cu	Cu	1·21	1·29	1·33	1·36	0·016
	Co	1·32	1·29	1·29	1·30	
Mn	Cu	1·63	1·75	1·86	1·99	0·017
	Co	1·80	1·81	1·81	1·81	
K_2O	Cu	6·46	5·12	3·55	2·72	0·15
	Co	4·83	4·28	4·41	4·33	
Ca+Mg	Cu	0·72	0·81	0·78	0·68	0·011
	Co	0·68	0·76	0·76	0·80	
P_2O_5	Cu	0·79	0·71	0·65	0·69	0·013
	Co	0·70	0·69	0·73	0·73	

Dry weight in g./2 pots.

Fe deficiency symptoms in angular degrees. Fe in μg., and Cu, Mn in $\log_{10}$ μg./g. dry matter. K_2O and P_2O_5 as % dry matter. Ca+Mg as m.equiv./g.

The value 1·12 g. for dry weight at Cu_0 is the mean dry weight of all plants not given Cu, averaged over all rates of Co; the other means given in the table are derived similarly.

Each value is the mean of twenty-four observations.

TABLE 4. *The effects of either cobalt or copper alone on oat tops*

	Effect of Cu at Co_0					Effect of Co at Cu_0			
	0	1	2	3	S.E.	0	1	2	3
Dry weight	1·13	1·17	1·07	0·73	0·06	1·13	1·12	1·14	1·06
Fe deficiency symptoms	0	0	0	19·6	7·2	0	5·0	11·8	16·4
Fe	126	112	104	84	4·6	126	115	125	117
Cu	1·23	1·32	1·37	1·38	0·032	1·23	1·22	1·19	1·20
Mn	1·67	1·73	1·85	1·96	0·033	1·67	1·64	1·65	1·56
K_2O	6·85	5·90	3·89	2·67	0·31	6·85	6·25	6·29	6·29
Ca+Mg	0·66	0·70	0·73	0·63	0·023	0·66	0·73	0·73	0·78
P_2O_5	0·79	0·74	0·64	0·64	0·026	0·79	0·79	0·80	0·78

Details of the units employed are given in Table 3. Each value is the mean of six observations.

Table 3 shows that both metals decreased dry weight and the concentrations of Fe and K in the plant tops. Dry weight was reduced to a similar extent by the application of either 25 m.equiv. Cu alone or 10 m.equiv. Co alone (Table 4).

Of these two treatments, Cu had much the more marked effect on both Fe and K concentrations, although the plants given 10 m.equiv. Co developed Fe deficiency symptoms, and those given 25 m.equiv. Cu did not. The effect of either metal in inducing these symptoms was much more marked in the presence than in the absence of the other (Co × Cu interaction significant at $P=0\cdot01$). There was no significant interaction between Co and Cu in their effects on dry weights, or on the concentrations of Fe and K.

The concentrations of Cu and Mn in the tops were increased by Cu and unaffected by Co application; the Co × Cu interaction was in neither case significant.

The Ca + Mg concentration was higher at Cu$_1$ and Cu$_2$, but lower at Cu$_3$ than at Cu$_0$ (at all rates of Co). Co-treated plants contained a much higher Ca + Mg concentration than controls.

Cu applied alone to the plants decreased phosphate concentration. Co applied alone had no effect on phosphate, but the reduction in P occurring at the higher rates of Cu application was countered by the addition of Co, as shown in Table 5.

TABLE 5. *The effects of cobalt and copper on phosphate concentration, percentage dry matter*

	Cu$_0$	Cu$_1$	Cu$_2$	Cu$_3$	Mean
Co$_0$	0·79*	0·74	0·64	0·64	0·70†
Co$_1$	0·79	0·68	0·62	0·66	0·69
Co$_2$	0·80	0·70	0·67	0·75	0·73
Co$_3$	0·78	0·71	0·68	0·74	0·73
Mean	0·79†	0·71	0·65	0·69	

* S.E. 0·026. † S.E. 0·013.

Insufficient material was available for individual Co analyses to be made, but analysis of the bulked digests showed that the Co$_3$ plants contained about 30 p.p.m. Co in the dried material.

There was a highly significant inverse correlation between the incidence of Fe deficiency symptoms (proportion of plants affected, expressed in angular degrees), and the concentration of iron (μg Fe/g. dry matter) in the plant tops:

$$r = -0\cdot693/95 \text{ degrees of freedom.}$$

Thus with increased addition of Co or Cu to the soil, progressively more plants developed Fe deficiency symptoms, and there was a simultaneous decrease in the concentration of Fe in the plant tops.

Barley

The symptoms usually observed in the leaves of plants given either Cu or Ni were white necrotic streaks, often accompanied by brown necrotic dots. In some plants the leaves became uniformly chlorotic or necrotic but Fe deficiency symptoms did not appear. In the absence of Ni, the incidence of the various symptoms, collectively (expressed in angular degrees), was 0, 0 and 27·1 at Cu$_0$, Cu$_1$ and Cu$_2$

respectively; in the absence of Cu it was 0, 34·3, 73·4 and 90·0 at Ni_0, Ni_1, Ni_2 and Ni_3 respectively. Cu was thus less effective than Ni in producing symptoms of toxicity.

Dry weight was greatly reduced by applying either metal (Table 6); the Cu × Ni interaction was not significant. Mortality was observed at the time of harvest in the treatments Cu_1Ni_3 (2 %), Cu_2Ni_2 (1 %) and Cu_2Ni_3 (12 %).

TABLE 6. *The effects of copper and nickel on barley*

		Rate of metal application				
Variable	Metal applied	0	1	2	3	S.E.
Dry weight	Cu	0·41	0·35	0·30	—	0·009
	Ni	0·48	0·41	0·31	0·21	0·011
Incidence of toxicity symptoms	Cu	49·4	60·4	71·4	—	2·6
	Ni	9·0	61·3	81·2	90·0	3·0
Fe	Cu	67·6	66·4	57·8	—	1·6
	Ni	75·0	66·5	58·7	55·4	1·9
Cu	Cu	1·11	1·19	1·45	—	0·026
	Ni	1·23	1·24	1·25	1·28	0·030
Mn	Cu	2·05	2·12	2·24	—	0·027
	Ni	2·08	2·13	2·14	2·18	0·031
Ni	Cu	1·82	2·20	2·54	—	0·045
	Ni	—	1·65	2·11	2·80	0·045
K_2O	Cu	7·22	6·34	6·01	—	0·14
	Ni	8·09	7·16	6·00	4·83	0·16
Ca + Mg	Cu	0·62	0·62	0·65	—	0·013
	Ni	0·61	0·62	0·61	0·68	0·015
P_2O_5	Cu	1·68	1·39	1·25	—	0·042
	Ni	1·51	1·62	1·50	1·12	0·049

Dry weight in $\log_{10}$ (1 + g./2 pots).

Incidence of chlorosis in angular degrees.

Fe in μg., and Cu, Mn, Ni in $\log_{10}$ μg./g. dry matter. K_2O and P_2O_5 as % dry matter. Ca + Mg as m.equiv./g.

The values given in the table were derived in the same way as those in Table 3. Except for Ni concentration, each Cu mean is based on twenty-eight, and each Ni mean on twenty-one, observations. For Ni concentration, each value is the mean of twenty-one observations.

Fe concentration was reduced by the application of either Cu or Ni, but tended to increase again at the higher rates of metal application.

Applying Cu alone to the plants slightly increased the concentrations of both Cu (Table 7) and Mn in the dried material, whereas Ni applied alone had the reverse effect. When, however, Cu and Ni were applied together, the Cu and Mn concentrations were greatly increased. Thus the plants at Cu_2Ni_3 contained twice as much Cu and Mn (μg./g. dry matter) as those at Cu_2Ni_0.

Ni concentration, too, was increased much more by the application of both Cu and Ni than by the application of Ni alone; the results are shown in Table 8. Untreated plants (Cu_0Ni_0) contained about 1·7 p.p.m. Ni in the dried material.

TABLE 7. *The effects of metal application on copper concentration, log_{10} (μg. Cu/g. dry matter)*

	Cu_0	Cu_1	Cu_2	Mean
Ni_0	1·17*	1·25	1·29	1·23†
Ni_1	1·15	1·20	1·38	1·24
Ni_2	1·12	1·14	1·49	1·25
Ni_3	1·00	1·17	1·66	1·28
Mean	1·11‡	1·19	1·45	

* s.e. 0·052. † s.e. 0·030. ‡ s.e. 0·026.

TABLE 8. *The effects of metal application on nickel concentration, log_{10} (μg. Ni/g. dry matter)*

	Cu_0	Cu_1	Cu_2	Mean
Ni_1	1·36*	1·66	1·91	1·65†
Ni_2	1·77	2·05	2·51	2·11
Ni_3	2·30	2·90	3·20	2·80
Mean	1·82†	2·20	2·54	

* s.e. 0·078. † s.e. 0·045.

K concentration was decreased by adding Cu, and especially Ni, to the soil: it was almost constant (5 % K_2O) at the highest rates of nickel application (Cu_0Ni_3, Cu_1Ni_3, Cu_2Ni_3 and Cu_2Ni_2).

The Ca+Mg concentration was slightly decreased by the addition of either Cu or Ni alone to the plants, but like Cu and Mn concentrations, it was considerably increased at Cu_2Ni_3.

Cu application reduced phosphate concentration, but the latter first increased, then subsequently decreased, as progressively more Ni was added to the soil. Phosphate, like K, tended to reach a constant minimum value at the highest rates of Ni application (about 1·1 – 1·2 % P_2O_5 at Cu_0Ni_3, Cu_2Ni_3 and Cu_2Ni_2).

1953 *experiment*

Oat

Iron deficiency symptoms appeared soon after transplanting in plants given $CuSO_4$.

Table 9 shows that the efficiency of Fe-EDTA in correcting symptoms of Fe deficiency decreased considerably during the experiment; moreover, symptoms

TABLE 9. *The incidence of iron deficiency symptoms (in angular degrees) in plants given 40 m.equiv. copper sulphate*

	m.equiv. Fe applied (as Fe-EDTA)		
Days from transplanting	0	1·5	3·0
5	53·6	26·2	7·3
10	61·5	61·0*	31·0*

* Widespread 'EDTA-necrosis' of the older leaves.

of necrosis appeared in the plants given Fe-EDTA. Similar symptoms were produced by either cupric- or ferric-EDTA, and consisted of either white or pale yellow irregular mottling, or a grey or brown discoloration spreading along the leaf from the tip. In a few plants, the necrosis took the form of white streaks, very similar to those observed in barley plants given Cu or Ni. No mortality occurred in the present experiment.

There was no significant interaction between $CuSO_4$ and Fe-EDTA in their effects on either dry weight or Fe concentration, and as mentioned earlier not all of the samples were analysed for Cu. The results are therefore given in the following table only for the effects of $CuSO_4$ in the absence of Fe-EDTA, and vice versa.

TABLE 10. *A comparison of the effects of copper sulphate and the cupric and ferric salts of EDTA, on oat plants*

	Control	40 m.equiv. Cu as $CuSO_4$	3 m.equiv. Cu as Cu-EDTA	3 m.equiv. Fe as Fe-EDTA
Dry weight (g.)	1·86	1·16 (±0·07)	1·18 (±0·08)	1·15 (± 0·07)
Cu (μg./g.)	11·9	31·0 (±1·2)	114·9 (±30·8)	11·5 (± 0·3)
Fe (μg./g.)	112	87 (±4·3)	390 (±21·8)	326 (±18·7)

Each value given is the mean of eight determinations.

Table 10 shows that all three treatments reduced dry weight by the same amount, the corresponding changes in the composition of the plant tops being:

$CuSO_4$, increase of 19·1 p.p.m. Cu, decrease of 25 p.p.m. Fe
Cu-EDTA, increase of 103 p.p.m. Cu, increase of 278 p.p.m. Fe
Fe-EDTA, no change in copper, Cu, increase of 214 p.p.m. Fe

At the same equivalent rates, Cu-EDTA increased Fe concentration more than did Fe-EDTA, as shown in Table 11.

TABLE 11. *The effects of copper sulphate and iron-EDTA on iron concentration, μg./g. dry matter*

m.equiv. applied	0	1·5	3·0	4·5	S.E.
Cu-EDTA	112	382	390	365	21·8
Fe-EDTA	112	307	326	—	18·7

DISCUSSION

Although appreciable fixation of Cu occurs in soils (see, for example, Steenbjerg & Boken, 1950), the addition of sufficiently large amounts of metal will eventually produce saturation. With the subsequent sudden increase in available Cu in the soil, the following effects were observed in these experiments:

(*a*) An abrupt increase in the concentration of Cu in the plant tops and a greater variation in the copper concentrations of replicate samples.

(*b*) An abrupt rise in mortality.

The following data, obtained with oat (1951), illustrate these points:

		Rate of copper application (m.equiv. $CuSO_4$/pot)			
		0	12·5	25	50
Cu concentration (μg./g.)	Mean	14	31	87	396
	Range	14–15	30–33	77–99	148–933*
Mortality (%)		0	1	33	83

* Only living plants were sampled.

Fe deficiency symptoms appeared in a few of the oat plants only in the 1951 experiments, and none of the crops showed any significant effect ($P=0{\cdot}01$) of Cu application on Fe concentration. The symptoms were, however, widespread in oat (1952), and their incidence was significantly correlated with the observed decrease in the concentration of Fe in the tops. Although Cu had a more marked effect than Co on Fe concentration in this experiment, Co was the more effective metal in inducing Fe deficiency symptoms. Fe concentration was also reduced in barley plants (1952) given Cu or Ni, but no symptoms of Fe deficiency appeared.

Barley plants given either Cu or Ni developed a white necrotic streaking of the leaves, and similar symptoms also appeared in some of the oat plants treated with either cupric- or ferric-EDTA. Other workers have shown that the symptoms are produced in oat plants by either excesses of Co or Ni (Macaulay Institute, 1952, 1953), or a *deficiency* of Fe (Vergnano & Hunter, 1952).

Hunter & Vergnano (1952) quote unpublished results which showed that Ni toxicity was alleviated by the application of Cu. In the present experiment, statistical analysis of the dry weights showed that the Cu × Ni interaction was not significant, i.e. Ni application reduced yield by a similar amount whether or not Cu was also added, and necrosis was actually more marked in the plants given both Cu and Ni than in those given Ni only. The former plants also contained unusually high concentrations of Cu, an effect invariably associated in the 1951 experiments with a marked decrease in yield (Table 1). The absence of such an effect here may be because Ni can in some way counter the toxic effect of Cu.

Co, Cu and Ni all reduced the concentration of total Fe in the plant tops, in agreement with the results obtained by Willis & Piland (1934), and Densch & Hunnius (1924) with Cu, and by Nemec & Babicka (1935) with Co salts. Immobilization of Fe would appear to have occurred in the soil or in the roots, but may also have occurred in the foliage, as suggested by Wallace (1947). Millikan (1947*a*) suggested that chlorotic leaves contain a higher concentration of Fe than green leaves, but he gives only two analyses in his paper, and of the four references cited in support of his suggestion, one (Hendrickson, 1924) gives no analytical data, and another (Rippel, 1923) concludes that 'Der prozentige Eisengehalt der chlorotischen und der sattgrünen Pflanzen ist völlig gleich'.

The high concentrations of Mn, Ca and Mg in Cu-treated plants may arise because the soil forms less stable complexes with these metals than with Cu

(Hasler, 1950). Antagonism between K and either Cu (Boratynski & Burstrom, 1937) or one of the other nutrients, e.g. Ca, may be responsible for the decreased K concentration in Cu-treated plants, whilst the decreased phosphate concentration can probably be attributed to precipitation of copper phosphate in the soil.

When Ni only was applied to barley, there was a decrease in the concentrations of all the nutrients (except Ni) in the dried material, but at the higher rates of both Cu and Ni the reverse effect tended to occur, suggesting respectively a decrease and then an increase in the ash: dry-weight ratio.

The increased Fe concentration in oat plants (1953) given Cu-EDTA may be due to the conversion of some of the Cu-EDTA ($K = 4{\cdot}2 \times 10^{-19}$) in the soil into Fe-EDTA ($K = 1 \times 10^{-25}$), which is then taken up by the plant. Plants given 3 m.equiv. Cu-EDTA contained 114·9 p.p.m. Cu, showed no mortality, and were of the same dry weight as plants given 40 m.equiv. $CuSO_4$ yet containing only 31 p.p.m. Cu. The apparent tolerance of the plant to high concentrations of copper when it is supplied as Cu-EDTA may be because the copper is assimilated mainly as slightly dissociating Cu-EDTA molecules, instead of as free cupric ions, the latter being presumably the more toxic.

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STUDIES ON THE SYNERGISTIC EFFECT OF PIPERONYL BUTOXIDE AND ISOBUTYLUNDECYLENEAMIDE ON PYRETHRINS AND ALLETHRIN

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(With 1 Text-figure)

Experiments are described to compare the toxicity of natural pyrethrins and allethrin, the completely synthetic homologue of cinerin I, and to show the effect of the two synergists, piperonyl butoxide and isobutyl undecyleneamide (IN 930) on both these active ingredients, using a measured-drop technique with *Musca domestica* L. the housefly, and a residual-film technique with *Cimex lectularius* L. the bed bug. In the conditions of the experiment, pyrethrins were shown to be twice as toxic as allethrin to flies, and 5·5 times as toxic as allethrin to bugs. The two synergists were tested at several ratios to the two insecticides, ranging from 1:1 to 20:1. The results were plotted as series of log. concentration/probit regression lines. These were parallel for the bug tests; but in the fly tests, the slope of the line increased with a rise in the proportion of synergist to insecticide. The estimated median lethal concentrations indicated, in all cases, that the toxicity increased with a rise in the ratio of synergist to insecticide, at least up to 20:1. However, the enhancement of toxicity was greatest for the smaller ratios and fell off as the ratio increased. Piperonyl butoxide was the more powerful synergist, increasing the potency of pyrethrins 5 times and allethrin 4 times to flies, and pyrethrins twice and allethrin 3 times to bugs, whereas IN 930 did not increase the potency of either ingredient more than twice to either test insect.

The addition of piperonyl butoxide to residual films of pyrethrins greatly prolonged their effectiveness; but an experiment designed to investigate the effect of the synergist on the stability of this insecticide showed that this action, if it exists, must be slight.

Introduction

Much has been written on the effect of synergists on pyrethrins, particularly piperonyl butoxide, which has been tested on a large variety of insects. This has been shown many times to be a powerful synergist for pyrethrins, but the only precise data on the relative effects of different combinations are in the work of Wachs (1947), Dove (1947), Hewlett (1951) and Blackith (1952).

Knowledge of the effect of synergists on allethrin is comparatively scanty. Piquett (1949), in preliminary experiments with dusts on cockroaches, and Moore (1950) using sprays against cockroaches, indicated that certain pyrethrum synergists had a similar effect on allethrin, but to a lesser degree. Jones, Schroeder & Incho (1950), using houseflies, compared the effects of synergists on pyrethrins and allethrin and found that, when either piperonyl butoxide, *n*-propyl isome or sesamin were mixed in equal quantities with each insecticide, the dose of allethrin required to give a 50% mortality was twice that of pyrethrum. In testing other insects they found great differences in the effect of the synergist with different species. They concluded that, in most cases, a synergist with allethrin gives a lower order of effectiveness than with a similar dose of pyrethrins, but that the effect of any particular synergist on allethrin cannot be predicted from existing results with pyrethrins. Gersdorff, Nelson & Mitlin (1951, 1952) worked with the synergists piperonyl butoxide, '264', *n*-propyl isome, benzyl propyl ether and sulfoxide. They showed, using house-flies and the turntable method, that in every case the synergist increased the toxicity of pyrethrins to a greater extent than that of allethrin but since they found allethrin to be 3 times as toxic as pyrethrins their pyrethrin mixtures were only slightly more toxic than the allethrin mixtures.

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Page & Blackith (1950), in experiments with pyrethrum synergists, showed that sesamin and isobutyl undecyleneamide increased the toxicity of the pyrethrins when combined in fixed ratios. They claimed that a maximum effect is obtained when the two are mixed in equi-molecular proportions, the mixture being 3 times as potent as the pyrethrins alone. Piperonyl butoxide differs from these two in that the maximum effect occurs with a 5:1 ratio. These effects were obtained both in flying and crawling insect assays using *Aëdes aegypti* L. and *Calandra granaria* L. From these results, they concluded that the synergist forms a loose molecular complex with the pyrethrins, which is 3 times as toxic as the pyrethrins alone. Miller, Pellegrini, Pozefski & Tomlinson (1952) found no evidence from cryoscopic and infra-red spectral studies of the formation of such a complex in mixtures of purified pyrethrins and piperonyl butoxide.

No investigators have used several combinations of synergist with pyrethrins and allethrin against more than one species, and this fact prompted the present investigations.

One of the greatest drawbacks of pyrethrins, in comparison with other insecticides, is their instability. It is well known that in thin films they are decomposed by heat and sunlight and rendered non-toxic in a matter of days. Early preliminary tests with allethrin gave good reason for believing that it would be of greater value in this respect than natural pyrethrins, because of the extra stability of thin films. Grannett, Connola & Lembach (1951) applied emulsions and acetone solutions of both substances to wooden panels. Allethrin panels gave a complete kill of house-flies after 3 months, whereas pyrethrins gave only 50% kill after a week. Busvine & Nash (1953) showed that allethrin residues from acetone solution on filter papers gave high kills of *Cimix lectularius* after 1 month while pyrethrins were ineffective after 3 days.

It has been reported (Dove, 1947; Chamberlain, 1950) that certain synergists, particularly piperonyl butoxide, increased the stability of pyrethrins in thin films, and several American workers upheld this view. It has been suggested (Page &

Blackith, 1950) that these claims are the result of confusion between the synergistic and stabilizing effects, the one not being taken into account when assessing the other. In experiments designed to separate the two effects, Hewlett (1951) and Blackith (1952) found no cause to uphold the claim that piperonyl butoxide stabilizes pyrethrin films.

MATERIALS AND METHODS

The following substances were used in this work:

DDT. Recrystallised sample, m.p. 108° C. Supplied by Geigy Co.

Pyrethrum concentrate: 25 %. Supplied by Stafford Allen and Co.

Allethrin, technical grade. Supplied by U.S. industrial chemicals. (This sample was shown by biological assay to be 80 % pure.)

Piperonyl butoxide, technical grade, 80 % pure.

Isobutyl undecyleneamide, technical grade (this substance will be referred to throughout as IN 930).

All the insecticides used in these tests were dissolved in Shell oil, P.31, a high boiling point refined paraffin oil of low volatility. The concentrations of all substances are expressed as % w/v, i.e. g./100 m. Both the pyrethrin and allethrin concentrates, and all solutions containing pyrethrins or allethrin were kept in the refrigerator in the dark when not in use.

The two insects selected for this work were the housefly, *Musca domestica* L. and the bed bug, *Cimex lectularius* L., as examples of two different types of insect, flying and crawling, drawn from among pests of medical importance.

The strain of flies used for this work was first collected from a natural habitat in Somerset, and has been kept in the laboratory of the London School of Hygiene and Tropical Medicine since September 1950. A second normal strain of flies which had been kept in the Hawthorndale Laboratories, Berkshire, for several years was obtained early in 1951, and the two were compared. There was no difference in the level of resistance of these strains to pyrethrins and allethrin, although the Somerset strain was slightly more resistant to DDT.

The strain of bed-bugs used had been kept at the London School of Hygiene and Tropical Medicine for about 15 years and was originally obtained from a natural infestation in London.

Rearing of test insects

Flies for experiment were reared in constant temperature rooms at 27° C. and 45–60 % R.H. Flies were kept in muslin cages, 6 in. cube, at a density of approximately 250 flies per cage. They were fed daily on equal parts of milk and water soaked on cotton wool pads, and were given a constant supply of sugar.

The larval medium consisted of two parts of guinea-pig meal (containing maize meal, wheat feed, dried milk, vitamins and yeast) and one part of each of sawdust and bran, mixed with water to give a moist medium. Eggs were placed in bowls of medium in large numbers, which were adjusted after 2 days to prevent over-crowding.

It was found to be no simple matter to produce standard flies for experimental purposes. Over-crowding of larval cultures produced undersized flies, while insufficient density of larvae caused poor cultures, moulds being able to flourish and prevent the larvae moving through the medium.

The bed bugs used were kept at a constant temperature of 27° C. and 80% R.H. in glass tubes, $2 \times 1\frac{1}{4}$ in., containing filter-paper, and covered by fine muslin. Bugs were fed weekly by attaching the tubes with an elastic band to the inner surface of the ear of a lop-eared rabbit. First-stage nymphs were isolated and placed in clean tubes every week, so that each tube contained bugs of one stage only. Adults emerged after 6 weeks, when any nymphs which had not reached the adult stage were removed.

Test methods. (1) *Flies*

When 4 days old, the flies were collected from cages in test-tubes and lightly anaesthetized with carbon dioxide. Each was treated individually with 0·4 mm.^{3} of solution on the dorsal side of the thorax, using a micrometer syringe. Flies were treated in batches of approximately thirty-five and placed in recovery cages. All operations were conducted at 27° C. Mortality counts were taken after 24 hr., the criterion of recovery being ability to stand and crawl. Partial recovery from 'knock down' occurred more frequently with pyrethrins than allethrin.

Advantages of test method

Some consideration was given to the accuracy of this test method as compared with the Peet-Grady Chamber, and the Campbell turn-table used so extensively in the U.S.A. The dose each fly received was clearly more consistent than in the Peet-Grady chamber, in which the pick-up of the insecticide depends so much on the activity of the flies. In the turn-table method, the flies are lying in all positions and are thus sprayed from different angles. Furthermore, the chilling which is employed not only affects metabolism, but causes condensation of moisture on the cuticle.

The following possible sources of error in the present method were investigated:

(1) Loss of insecticide by contact with cage.
(2) Duration of stupefaction by carbon dioxide.
(3) Effect of crowding in recovery cages.
(4) Order of collection of batches of flies from stock cages.

None of these factors had any pronounced effect and, so far as possible, were maintained constant throughout.

Effect of sex on susceptibility

In preliminary experiments, using both male and female flies the males proved much more susceptible than females to both pyrethrins and allethrin. The results for each sex therefore could either be recorded separately, and two regression lines drawn, or the male and female mortalities could be averaged, resulting in one non-linear regression line. It was decided that female flies only would be used in this work.

Test methods. (2) *Bugs*

Bugs were used for experiment 3 days after the third weekly feed of their adult life. Barnes (1946), investigating the effects of DDT on bed bugs, found that at this stage males and females were equally susceptible. The syringe method used for flies was found unsuitable for bugs, since drops of oil solution did not spread over the entire area of the thorax, and were lost, either on the container, or by contact with other bugs. The method of contact with a treated surface was therefore employed, and preliminary experiments showed that the most suitable film was formed by the application to filter-paper of solutions of active ingredient and synergist in P. 31, added to ether in the ratio of 1:2. This gave a constant quantity of oil (0·33 mg./cm.^{2}), at every concentration. 1 ml. of solution was applied to a Whatman No. 1 filter-paper (11 cm. diam.), from a hypodermic syringe, as evenly as possible, and the papers dried for about 2 hr. before using, allowing the ether to evaporate and the oil solution to spread evenly. Bugs were confined on the papers for 24 hr. at 30° C., under inverted funnels, and then removed to clean containers. Mortalities were assessed after 6 days recovery at 30° C., the criterion of death being inability to stand and crawl.

EFFECTS OF SYNERGIST AT VARIOUS RATES

The experiments which form the first part of this work were designed to give a series of log. concentration/probit regression lines for each of the active ingredients, pyrethrins and allethrin, combined with both the synergists, piperonyl butoxide and isobutyl undecyleneamide (IN 930), using both test insects.

The concentrations of active ingredients used were chosen at approximately logarithmic intervals which ranged between 0·0075 and 3% in experiments with flies, and between 0·03 and 2% in experiments with bugs. Synergists were combined with the active ingredients at ratios of 1:1, 5:1, 10:1 and 20:1. Each mixture was tested on approximately thirty-five flies and twenty bugs and each curve was based on at least two replicate tests at each concentration. Control experiments showed that mortalities caused by the synergists alone were negligible to both test insects at the concentrations used.

RESULTS

Mortalities, expressed as probits, were plotted against log. concentrations of the active ingredient, and in every case these were found to be linearly related over the range of concentrations tested. The regression equations were computed, and are given in Tables 1 and 2. With the technique employed of applying a measured

TABLE 1. *Analysis of results. Flies: measured-drop technique*

			χ^2	b	Log L.D. 50	L.D. 50 % soln.
Pyrethrins	—	$Y = 8{\cdot}007 + 2{\cdot}78x$	0·820, $n=3$	2·78 ± 0·259	−1·081 ± 0·0270	0·083
Pyrethrins with piperonyl butoxide	1:1	$Y = 10{\cdot}384 + 3{\cdot}85x$	4·284, n=2	3·85 ± 0·363	−1·397 ± 0·0244	0·040
Pyrethrins with piperonyl butoxide	5:1	$Y = 14{\cdot}553 + 5{\cdot}87x$	0·348, $n=1$	5·87 ± 0·502	−1·628 ± 0·0142	0·024
Pyrethrins with piperonyl butoxide	10:1	$Y = 14{\cdot}996 + 5{\cdot}99x$	0·521, n=2	5·99 ± 0·563	−1·670 ± 0·0176	0·021
Pyrethrins with piperonyl butoxide	20:1	$Y = 15{\cdot}738 + 5{\cdot}59x$	0·201$n=1$	5·95 ± 0·519	−1·805 ± 0·0145	0·016
Pyrethrins with IN 930	1:1	$Y = 7{\cdot}790 + 2{\cdot}52x$	1·911, $n=2$	2·52 ± 0·308	−1·107 ± 0·0282	0·078
Pyrethrins with IN 930	5:1	$Y = 9{\cdot}632 + 3{\cdot}74x$	0·037, $n=2$	3·74 ± 0·339	−1·238 ± 0·0199	0·058
Pyrethrins with IN 930	10:1	$Y = 9{\cdot}095 + 3{\cdot}04x$	2·481, $n=2$	3·04 ± 0·322	−1·348 ± 0·0243	0·045
Pyrethrins with IN 930	20:1	$Y = 10{\cdot}692 + 3{\cdot}97x$	0·616, $n=2$	3·97 ± 0·421	−1·434 ± 0·0211	0·037
Allethrin	—	$Y = 8{\cdot}517 + 4{\cdot}50x$	1·176, $n=1$	4·50 ± 0·352	−0·781 ± 0·0131	0·17
Allethrin with piperonyl butoxide	1:1	$Y = 12{\cdot}075 + 6{\cdot}89x$	0·718, $n=2$	6·88 ± 0·478	−1·027 ± 0·0125	0·094
Allethrin with piperonyl butoxide	5:1	$Y = 12{\cdot}289 + 6{\cdot}07x$	1·064, $n=2$	6·07 ± 0·444	−1·200 ± 0·0123	0·063
Allethrin with piperonyl butoxide	20:1	$Y = 14{\cdot}174 + 6{\cdot}61x$	2·096, $n=1$	6·61 ± 0·436	−1·387 ± 0·0110	0·041
Allethrin with IN 930	1:1	$Y = 9{\cdot}259 + 4{\cdot}48x$	1·515, $n=1$	4·48 ± 0·554	−0·951 ± 0·0202	0·11
Allethrin with IN 930	5:1	$Y = 11{\cdot}873 + 6{\cdot}67x$	3·428, $n=1$	6·67 ± 0·670	−1·030 ± 0·0170	0·093
Allethrin with IN 930	10:1	$Y = 12{\cdot}042 + 6{\cdot}55x$	3·060, $n=2$	6·55 ± 0·564	−1·075 ± 0·0159	0·084
Allethrin with IN 930	20:1	$Y = 13{\cdot}065 + 6{\cdot}96x$	0·671, $n=1$	6·96 ± 0·736	−1·158 ± 0·0152	0·069

TABLE 2. *Analysis of results. Bed-bugs: residual film technique*

			χ^2	b	Log L.D. 50	M.L.D. (% soln.)
Pyrethrins	—	$Y = 17{\cdot}187 + 5{\cdot}43x$	1·422, $n=2$	5·43 ± 0·551	−2·246 ± 0·0151	0·17
Pyrethrins with piperonyl butoxide	1:1	$Y = 16{\cdot}196 + 4{\cdot}90x$	0·829, $n=1$	4·90 ± 0·841	−2·285 ± 0·0202	0·16
Pyrethrins with piperonyl butoxide	10:1	$Y = 21{\cdot}829 + 6{\cdot}80x$	1·046, $n=1$	6·80 ± 0·830	−2·474 ± 0·0173	0·10
Pyrethrins with piperonyl butoxide	20:1	$Y = 19{\cdot}920 + 5{\cdot}79x$	2·710, $n=1$	5·79 ± 0·764	−2·579 ± 0·0194	0·078
Pyrethrins with IN 930	1:1	$Y = 20{\cdot}323 + 6{\cdot}50x$	3·770, $n=2$	6·50 ± 0·673	−2·359 ± 0·0144	0·13
Pyrethrins with IN 930	10:1	$Y = 14{\cdot}470 + 3{\cdot}79x$	1·009, $n=2$	3·79 ± 0·475	−2·497 ± 0·0226	0·096
Pyrethrins with IN 930	20:1	$Y = 15{\cdot}774 + 4{\cdot}22x$	0·600, $n=1$	4·22 ± 0·601	−2·555 ± 0·0223	0·084
Allethrin	—	$Y = 19{\cdot}889 + 9{\cdot}81x$	0·054, $n=1$	9·81 ± 1·150	−1·518 ± 0·0121	0·91
Allethrin with piperonyl butoxide	1:1	$Y = 18{\cdot}800 + 7{\cdot}90x$	—	7·90 ± 1·375	−1·748 ± 0·0189	0·54
Allethrin with piperonyl butoxide	10:1	$Y = 20{\cdot}631 + 7{\cdot}99x$	0·449, $n=1$	7·99 ± 0·955	−1·956 ± 0·0189	0·331
Allethrin with IN 930	1:1	$Y = 17{\cdot}507 + 7{\cdot}39x$	—	7·39 ± 0·949	−1·692 ± 0·0148	0·61
Allethrin with IN 930	10:1	$Y = 18{\cdot}958 + 8{\cdot}37x$	—	8·37 ± 1·139	−1·667 ± 0·0148	0·66

drop directly to the body of the test insect, comparison of the log. concentration/probit regression lines shows pyrethrins to be more toxic than allethrin to flies at low concentrations, and equally toxic at high concentrations (0·5 % solution of either gives 99 % mortality). At the level of 50 % kill, allethrin proves to be half as toxic as pyrethrins. When tested by exposure to a residual film, there is considerably more difference between the two toxicities to bugs, pyrethrins proving to be 5·3 times as toxic as allethrin. In this case also, the slope of the curve for allethrin is much steeper than that for pyrethrins, but since there is so large a gap between the toxicities of the two substances the lines do not cross.

Reference to values of *b* in Table 1 shows that in tests with flies, a mixture of synergist and active ingredient in the ratio of 5:1 gives a regression line of greater slope than the active ingredient alone, but that higher ratios do not further increase the slope. Table 2 shows no similar increases in the values of *b* in experiments with bugs.

The median lethal doses of each mixture, as calculated from the regression equations, are given in Tables 1 and 2. The ratios

$$\frac{\text{Median lethal dose of active ingredient alone}}{\text{Median lethal dose of active ingredient:synergist at a fixed ratio}}$$

were calculated to give a measure of the increase in potency which results from the addition of the synergist at different ratios, and can be used to compare the effectiveness of each synergist with pyrethrins and allethrin. Fig. 1 shows graphically the

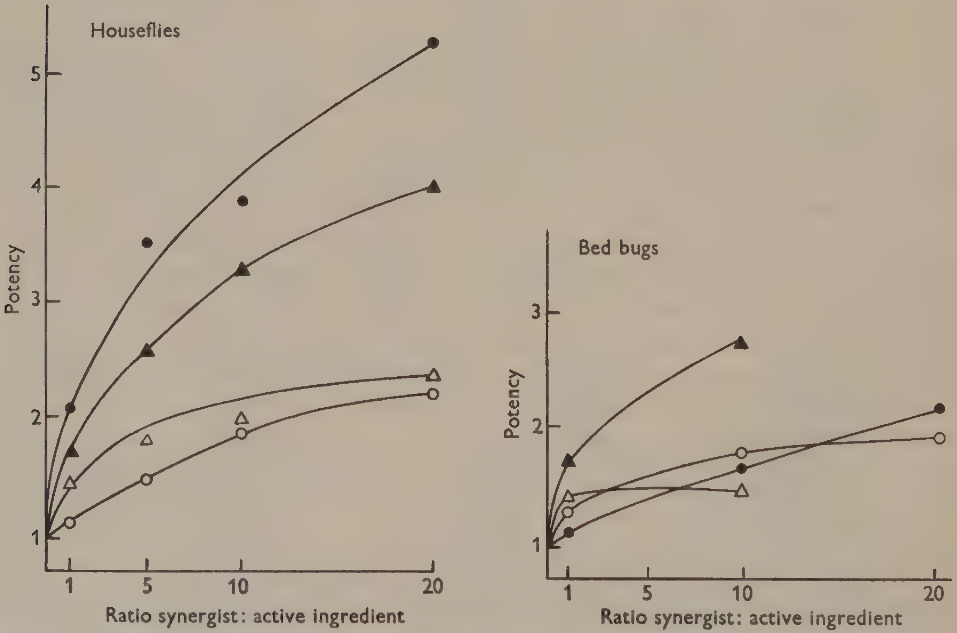

Fig. 1. Increase in potency with increasing ratio of synergist. ● pyrethrins + piperonyl butoxide, ○ pyrethrins + IN930, ▲ allethrin + piperonyl butoxide, △ allethrin + IN930.

connexion between these two quantities, and Table 3 shows the ratios of the M.L.D.'s at a 10:1 ratio of synergist: active ingredient. Piperonyl butoxide is shown to be more effective as a synergist with pyrethrins against flies, but more effective with allethrin against bugs. IN 930 is almost equally effective with both active ingredients against both insects.

TABLE 3. *Ratios of* M.L.D.*'s at a ratio of synergist: active ingredient of* 10:1

	Flies		Bugs	
	Pyrethrins	Allethrin	Pyrethrins	Allethrin
Piperonyl butoxide	4	3.25	1·75	2·75
IN 930	1·75	2	1·75	1·5

The data available can be rearranged to study the effect of combinations of constant dose of synergist with the active ingredient instead of fixed ratios, and Table 4 shows that in the case of piperonyl butoxide with pyrethrins tested on flies, log. dose of pyrethrins is linearly related to probit mortality for four different concentrations of synergist. There is no significant increase in slope, with increase of concentration of synergist.

Table 4. *Analysis of the effect of constant doses of piperonyl butoxide on pyrethrins*

% piperonyl butoxide		χ^2	b	Log L.D.$_{50}$
—	$Y = 8{\cdot}007 + 2{\cdot}78x$	0·820, $n=1$	2·78 ± 0·259	−1·081 ± 0·0270
0·075	$Y = 9{\cdot}787 + 3{\cdot}13x$	2·952, $n=2$	3·13 ± 0·336	−1·529 ± 0·0290
0·15	$Y = 9{\cdot}907 + 2{\cdot}88x$	0·086, $n=2$	2·88 ± 0·444	−1·703 ± 0·0441
0·3	$Y = 12{\cdot}059 + 3{\cdot}86x$	0·463, $n=2$	3·86 ± 0·422	−1·830 ± 0·0274
0·6	$Y = 12{\cdot}475 + 3{\cdot}81x$	0·593, $n=2$	3·81 ± 0·461	−1·961 + 0·0273

THE EFFECT OF PIPERONYL BUTOXIDE ON STABILITY OF PYRETHRINS

This subject has caused a great deal of controversy, and two types of experiments to study it are here described.

Effect of addition to residual films. Residual films were prepared by applying solutions in ether of pyrethrins, allethrin and mixtures of each with piperonyl, butoxide in a 10:1 ratio to filter-paper at a rate of 0·1 mg./cm.^{2} of active ingredient. Papers were kept in diffuse daylight and tested, using bed bugs as described earlier, at periods up to 12 months after treatment. The results are shown in Table 5.

The effect of addition to solutions. This experiment was designed to test the hypothesis that piperonyl butoxide increases the stability as well as the toxicity of pyrethrin solutions, and an attempt was made to separate the synergistic and stabilizing effects. The plan was to compare the effect of ageing, under conditions of diffuse daylight and room temperature, on a mixture of pyrethrins and piperonyl butoxide with the effect on a similar mixture whose constituents had been exposed separately. The toxicity of the solutions was tested at intervals by application to houseflies as described above.

TABLE 5. *The effect of the addition of piperonyl butoxide on the residual activity of pyrethrin and allethrin films*

		Percentage mortality of bugs				
	mg./cm.²	10 days	1 month	3 months	6 months	1 year
Pyrethrins	0·1	100	18	0	—	—
Pyrethrins + piperonyl butoxide	0·1 + 1·0	100	100	100	85	0
Allethrin	0·1	—	100	28	0	—
Allethrin + piperonyl	0·1 + 1·0	—	100	98	81	0

Solutions were placed for keeping in small Petri dishes, covered by transparent cellophane, the film of solution being 0·16 cm. in depth. These dishes were prepared in sets of three, one containing the mixture comprising 0·025 % pyrethrins and 0·125 % piperonyl butoxide (5 : 1 ratio) and the other two 0·5 % pyrethrins and 0·25 % piperonyl butoxide respectively, for mixing on the day of test. The solutions were kept in the refrigerator and one set removed each fortnight. Further exposure resulted in a set of solutions which had been exposed for 8, 12 and 14 weeks. All these were tested on the same day, to ensure comparable results, and the whole experiment repeated twice. The results are shown in Table 6.

TABLE 6. *Comparison of the effect on the mortality of flies of pyrethrin-piperonyl butoxide mixtures whose constituents were mixed before and after 'ageing'*

p_1 = percentage killed by mixture aged as a whole; p_2 = percentage killed by mixture aged separately. * = significant difference; $P = 0·05$.

Weeks aged	Male flies			Female flies		
	p_1	p_2	$p_1 - p_2$	p_1	p_2	$p_1 - p_2$
8	96	94	2 ± 5·5	21	31	−9 ± 10·8
	94	100	−6 ± 4·4	21	14	7 ± 8·9
	95	90	5 ± 5·7	15	37	−22 ± 9·7*
12	87	54	33 ± 12·0*	38	14	24 ± 12·4
	88	69	20 ± 10·0*	6	2	3 ± 4·5
14	74	38	35 ± 12·5*	17	6	11 ± 8·2
	71	40	30 ± 11·8*	16	8	8 ± 7·7
	37	31	6 ± 10·8	13	4	8 ± 5·8

DISCUSSION

When comparing the toxicities of pyrethrins and allethrin to flies, Stoddard & Dove (1949) and Nash (1950) both found that probit lines were steeper for allethrin than pyrethrins. The results shown in the present investigations agree with this finding. Agreement is not reached, however, on the ratio of the toxicities of these two, allethrin being half as toxic as pyrethrins in this work, and 3 times as toxic according to Gersdorff (1949), but equally toxic according to Moore (1950). These differences may well be attributed to the difference in experimental techniques. The low toxicity of allethrin to bugs in comparison with pyrethrins upholds the views of other workers that allethrin compares unfavourably with pyrethrins for most insects tested other than the housefly.

The importance has been emphasized of the need for a set of data which would give precise information on the effects of synergists on both natural pyrethrins and the completely synthetic allethrin. It is shown that piperonyl butoxide is a more active synergist than IN 930 to both active ingredients, but that the extent of the difference is not the same for each test insect. The effect of piperonyl butoxide on the toxicity of pyrethrins is greater for flies than for bugs, but there is little difference in the activity of IN 930 to the two insects. The relative increase in the toxicity of alllethrin, due to the addition of either synergist, is slightly higher for flies than bugs. This upholds the view of Jones *et al.* (1950), that the effect of synergists on allethrin cannot be predicted from data concerning pyrethrins. Quantitative comparisons between the effects on these two insects must, however, be made with caution. Hewlett (1951) found that the pyrethrum equivalent of a mixture could vary both with the species of insect, and with the method of application. Page & Blackith (1949, 1950) found that change of test method made a difference to the relative potency with IN 930 but not with sesamin. Only if the two insects could be treated by the same technique would the results be strictly comparable.

Blackith (1952) has stated that addition to pyrethrins of piperonyl butoxide beyond a molecular ratio of 5:1, and of IN 930 beyond a ratio of 1:1, does not further increase the toxicity. The data of Dove (1947) and Hewlett (1951), however, show that the toxicity can be increased by the addition of much greater quantities of synergist. The data in this work support this view. There is an increase in mortality with addition of either synergist to either active ingredient up to a ratio of 20:1, although the increase becomes progressively smaller with the addition of greater quantities of synergist. Since these substances are toxic at high concentrations (Dove, 1947), the difficulty of separating the synergistic and toxic properties would complicate the investigation of synergists at a ratio greater than 20:1.

The results of the first stability experiment show that the addition of piperonyl butoxide to pyrethrins in residual films lengthens the period of time over which they remain toxic. The mixture is more toxic at the outset than the pyrethrins alone, however, and therefore might be expected to give a higher kill throughout. This experiment therefore does not in any way prove that the synergist has a stabilizing effect on pyrethrins. It does show that for practical purposes the addition of synergist greatly increases the length of useful life of a pyrethrin film.

The results of the second experiment lead to no very definite conclusion. There appears to be slight protection by the synergist, for a higher proportion of flies was killed by the mixture of constituents aged together in thirteen cases, as compared with three cases in which the constituents aged separately killed more. Four of the former differences reached significance ($P=0\cdot05$), as compared with one of the latter. Generally, however, the differences are slight and the effect, if present, cannot be very pronounced. It does not seem to be of practical importance.

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ANALYSIS OF VIRUS LOCAL LESION EXPERIMENTS

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Calculations based on variation of lesion counts about means within the range 7 to 260 per half-leaf show that the standard deviation may be rendered independent of the mean by a transformation $\log(x+12)$ for half-leaf experiments in bean, tobacco and *Nicotiana glutinosa* and $\log(x+21)$ for whole-leaf experiments in bean.

Half-leaf experiment designs are superior to those using whole-leaves.

Balanced Incomplete Block designs are more efficient than Randomized Blocks or comparisons using a common standard.

The use of covariance to remove the effect of leaf susceptibility gives increased accuracy over the treatment-standard comparison if used with designs allowing removal of both plant variation and that due to leaf age.

INTRODUCTION

Necrotic local lesions produced by certain plant viruses on appropriate hosts have been widely used to estimate relative infectivities since Holmes (1929) showed that the number of lesions produced varied with the strength of the inoculum. Various experiment designs for infectivity measurements and methods of analysis of results have been suggested. Factors that influence the susceptibility of plants have been studied by several workers. The above work has been summarized by Bawden (1950).

A characteristic feature of local lesion counts is that of increasing variability with increasing number of lesions. Preparations of different infectivities not only produce different mean lesion counts, but the variation about the means in each case also differ. To enable valid comparisons to be made between the mean numbers of lesions produced by such preparations the statistical technique of Analysis of Variance is used. This technique assumes homogeneity of variation for all preparations independent of the size of the observed mean.

To conform with this basic assumption Kleczkowski (1949) has shown that analysis of the lesion counts on a logarithmic scale ($\log x+c$) satisfies this requirement. In the present investigation data from a number of trials* have been used to study the variation of lesion numbers in bean, tobacco and *Nicotiana glutinosa* L.

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* The lesion counts have been deposited at the Plant Diseases Division Library and are available on request.

plants in relation to the means with a view to obtaining a suitable transformation for these host plants. Using the transformed counts the relative accuracy of various possible experimental designs has been obtained by examining the relative size of the errors resulting from different designs superimposed on the same batch of experimental data. In this way the extent to which plant, leaf, and leaf side variation is removed from the error is ascertained.

MATERIALS AND METHODS

The host-virus combinations used were: French bean (*Phaseolus vulgaris* L. var. Black Valentine) with a tobacco necrosis virus, tobacco (*Nicotiana tabacum* L. var. White Burley) with turnip mosaic virus and *N. glutinosa* with tobacco mosaic or tomato spotted wilt viruses.

The various experiments listed in Table 1 were designed to give different mean lesion numbers for otherwise comparable groups of plants within the experiments by the use of different operators (Exp. 1); different abrasives, carborundum powder grade 4F or the diatomaceous earth 'Dicalite' (Exp. 3); or no abrasive and 'Dicalite' with different sap dilutions (Exp. 5); or keeping plants in the dark for 24 hr., removing growing points at different times before inoculation and inoculation at different times of the day (Exp. 4).

VARIATION OF LESION NUMBERS

To examine the variation of lesion numbers between half-leaves within leaves for bean, tobacco and *N. glutinosa*, and half-leaves and whole-leaves within plants for bean the regression of the means, m, on the appropriate standard deviations, s, have been calculated by the method of least squares. From the regression line for each experiment a value of the slope, b, and the intercept on the m axis, c, has been obtained.

The relation between the mean and standard deviation of units within each experiment is widely different from group to group as shown by the large variation in values of b and c (Tables 1 and 2). The average values of mean and standard deviation, however, exhibit a marked linear trend (Table 3). The use of different abrasives (Exps. 3 and 5), inoculation at different times of the day (Exp. 4) (Matthews, 1953), removal of growing points at different times before inoculation, and exposure to 24 hr. darkness (Exp. 4) all caused differences in the number of lesions produced by the same inoculum. However, there is a corresponding change in the standard deviations which maintains the linear trend. Thus for batches of half leaves (and whole leaves in bean) inoculated with the same virus preparation and producing a given mean, the mean and the standard deviation bear a constant relation.

The close agreement between the regression lines for half-leaves within leaves for bean, tobacco and *N. glutinosa* shows that the relation of mean to standard deviation is not dependent on any particular host-virus combination.

TABLE 1. *Variation of local lesion numbers: tobacco necrosis virus on bean*

(n = number of observations; m = mean of lesion numbers; s = mean of standard deviations; b = slope; c = intercept on m axis.)

	Half-leaves within leaves*					Half-leaves within plants					Whole-leaves within plants				
Exp.	n	m	s	b	c	n	m	s	b	c	n	m	s	b	c
1A	48	95·6	16·7	0·101	69	46	77·4	16·9	0·133	49	46	154·8	21·3	0·083	100
	44	57·6	12·6	0·249	−7	—	—	—	—	—	—	—	—	—	—
1B	48	62·1	13·0	0·181	9	50	89·0	22·7	0·153	59	50	178·0	33·9	0·086	215
	52	113·4	22·0	0·061	247	—	—	—	—	—	—	—	—	—	—
2	217	7·3	2·5	0·220	4	104	7·5	3·4	0·310	4	104	15·0	5·1	0·264	4
3A	40	16·7	4·6	0·215	5	60	19·1	7·8	0·266	10	60	38·1	10·7	0·132	43
	40	17·9	6·2	0·367	−1	—	—	—	—	—	—	—	—	—	—
	40	22·0	6·4	0·292	0	—	—	—	—	—	—	—	—	—	—
3B	40	34·0	7·8	0·292	−7	60	44·6	12·7	0·250	6	60	89·2	16·3	0·083	107
	40	34·4	7·4	0·091	48	—	—	—	—	—	—	—	—	—	—
	40	65·2	15·3	0·215	6	—	—	—	—	—	—	—	—	—	—
4A	48	17·0	4·8	0·219	5	48	28·6	9·1	0·317	0	48	57·7	13·3	0·257	−6
	48	40·8	9·7	0·300	−9	—	—	—	—	—	—	—	—	—	—
4B	44	44·4	5·6	0·116	4	46	42·2	9·8	0·087	70	46	85·2	15·5	0·383	45
	48	40·7	8·2	0·132	22	—	—	—	—	—	—	—	—	—	—
4C	48	62·5	9·0	0·271	−29	45	76·9	14·8	0·187	2	44	157·4	24·0	0·141	12
	40	98·1	13·6	0·072	91	—	—	—	—	—	—	—	—	—	—
4D	48	37·8	6·3	0·112	19	47	35·3	8·5	0·217	4	46	73·9	13·9	0·264	−21
	44	34·2	5·8	0·096	26	—	—	—	—	—	—	—	—	—	—
4E	46	66·3	12·4	0·161	11	46	62·7	12·9	0·311	21	46	121·3	19·1	0·172	−10
	46	54·3	7·8	0·135	3	—	—	—	—	—	—	—	—	—	—
5A	58	14·8	3·7	0·037	73	29	14·9	4·9	0·094	38	29	29·7	6·4	0·115	25
5B	56	13·1	4·0	0·222	5	28	13·2	5·0	0·338	2	28	26·1	6·2	0·332	−7
5C	58	8·3	2·7	0·312	0	29	8·3	3·6	0·521	−1	29	16·6	5·5	0·501	−6
5D	68	60·2	12·0	0·222	−6	34	60·1	16·1	0·211	16	34	123·2	24·1	0·153	34
5E	58	43·9	9·0	0·152	15	29	43·9	9·9	0·164	17	29	87·7	9·2	0·053	84
5F	64	35·4	8·3	0·163	15	32	35·6	10·1	0·036	244	32	70·5	16·0	0·260	−9

* For variation within leaves, except in expt. 2, each tray of twenty to thirty plants has been regarded as a group. Within plant variation has been calculated on the whole experiment.

From the regression lines in Table 3 it is clear that the transformations $\log(x+12)$ for half-leaf experiments and $\log(x+21)$ for whole-leaf experiments are satisfactory and preferable to the use of a different transformation for each experiment.

EXPERIMENT DESIGN

Three forms of basic design are considered. The plant may be regarded as the block comprised of whole-leaf units (whole-leaves within plants), or of half-leaf units (half-leaves within plants), or the leaf itself taken as a block comprising two half-leaf units (half-leaves within leaves). For experiments involving tobacco or *N. glutinosa* as host plants the last form only is applicable because of age differences between leaves of the same plant.

TABLE 2. *Variation of local lesion numbers*

(n=number of observations; m=mean of lesion numbers; s=mean of standard deviations; b=slope; c=intercept on m axis.)

Exp.	Leaf age	Half-leaves within leaves n	m	s	b	c
		Turnip mosaic virus on tobacco.				
1	1	43	56·1	11·1	0·173	8
	2	45	65·6	11·3	0·086	67
	3	42	35·5	7·9	0·133	24
2	1	47	15·7	4·5	0·060	59
	2	47	29·2	5·8	0·121	19
	3	47	31·0	6·0	0·003	2156
3	1	46	30·3	5·7	0·145	9
	2	46	40·3	7·7	0·034	184
	3	46	30·9	7·2	0·221	2
4*	1	48	15·8	5·2	0·422	−3
	2	46	24·3	5·6	0·167	9
	3	47	27·7	6·9	0·142	20
		Tobacco mosaic virus on *N. glutinosa*.				
1	1	49	16·3	5·8	0·110	37
	2	49	21·9	5·1	0·068	53
	3	49	24·0	5·9	0·075	54
	4	49	18·3	4·7	0·236	2
	5	49	16·2	4·0	0·161	8
	6	49	14·0	4·1	0·033	109
2	1	24	87·7	13·8	0·045	221
	2	24	121·2	26·3	0·220	−2
	3	24	155·2	21·2	−0·016	−1449
	4	24	173·8	34·9	0·159	46
	5	24	220·8	46·7	0·036	1056
	6	24	259·0	37·9	0·026	1198

* Tomato spotted wilt virus.

TABLE 3. *Variation of local lesion numbers. Regression lines of means over all experiments*

(n=number of observations; m=mean of lesion numbers; s=mean of standard deviations; b=slope; c=intercept on m axis.)

Host	Variation	n	m	s	b	5 % limits of b	c	5 % fiducial limits of c*
Bean	Half-leaf within leaf	27	44·4	8·8	0·156	±0·024	11·86	4·27 to 22·27
	Half-leaf within plant	16	41·2	10·5	0·197	±0·033	12·24	4·64 to 22·86
	Whole-leaf within plant	16	82·8	15·0	0·145	±0·015	21·13	2·74 to 49·52
Tobacco	Half-leaf within leaf	12	33·5	7·1	0·141	±0·029	16·36	7·86 to 29·27
N. glutinosa	Half-leaf within leaf	12	94·0	17·5	0·166	±0·034	11·88	−6·00 to 38·90

* Obtained directly from 5 % limits of b with m fixed.

If the number of treatment preparations to be compared is a factor of the number of units available in the block a Randomized Block design (Yates, 1936) may be used to remove block variation. When the number of treatments exceeds the number of units in the block, however, a Balanced Incomplete Block design (Yates, 1936) can be applied or larger blocks formed by amalgamation.

For half-leaf designs, and in the case of beans, for whole-leaves, the technique of analysis of covariance may be used by applying a common standard preparation to one half of each leaf, the other half receiving the treatment preparation. Assuming leaf susceptibility proportional to the transformed numbers of lesions produced by the standard preparation, it is measured as a constant multiple of the amount by which the transformed count of the standard on a leaf exceeds the average of such values for the experiment. Thus if x and y denote the transformed counts of standard and treatment preparations on a leaf and $\bar{x}$ the mean of the transformed standard counts within the experiment we may write

$$y = m + t + k(x - \bar{x}) + e,$$

where m denotes the general level of the experiment, t the effect of the treatment preparation, $k(x-\bar{x})$ the measure of leaf susceptibility and e a random error term. The computational technique and method of determining k, the strength of the correction for various experiment designs, is fully covered by Cochran & Cox (1950) and Snedecor (1946), while Finney (1946) gives a method of obtaining the average error variance of all paired treatment comparisons. The half-leaf transformed count for each treatment preparation is adjusted by subtracting its corresponding measure of leaf susceptibility. The mean of these adjusted values for a particular treatment combination measures the treatment effect.

A measure of the relative efficiencies of the different experimental arrangements is obtained by superimposing the designs on the same group of plants inoculated as uniformly as possible under experimental conditions, and comparing the resultant variances of treatment comparisons. As there are no treatment differences, the differences between any two treatment means is in all cases a measure of zero effect, and the variances of such differences obtained from the designs a direct measure of their precision. For two designs containing different numbers of replications the comparison is carried out on a per-plant basis, the observations for each treatment being taken as equal in number to the total of treatment observations occurring on any one plant for each design.

Beans

Thirty-two bean plants adjacently situated within the trial were taken from each of Exps. 4D and 5D (Table 1) and each group arranged in different experimental arrangements designed as experiments involving the comparison of four treatment preparations. From Exp. 3A designs involving six treatment preparations on thirty plants were similarly considered. The arrangements were as follows:

Whole-leaf comparisons (lesion counts transformed log $(x+21)$)

Treatment versus Standard (T*vS*). Treatments were applied to one whole leaf of a plant and a common standard to the other. Exps. 4 D and 5 D, four treatments, eight plants per treatment; Exp. 3 A, six treatments, five plants per treatment. Each treatment-standard pair was analysed as a Randomized Block design, each plant being regarded as a block.

Randomized Block analysed by Covariance (*R.B.Cov.*). Plants were arranged in groups, the number in each group being equal to the number of treatments. Each treatment was applied once in each group on one leaf of a plant with a standard on the other leaf. The treatments were assigned randomly to plants within groups. Exps. 4 D and 5 D, eight groups of four plants; Exp. 3 A, five groups of six plants. The data were analysed as a Randomized Block design with covariance correcting for plant susceptibility within each block.

Balanced Incomplete Block design (*BIBD*). All possible combinations of treatments taken two at a time were randomly assigned to the plants, each treatment occupying a whole-leaf. Exps. 4 D and 5 D, four replications of six plants; Exp. 3 A, two replications of fifteen plants. The data were analysed as a replicated Incomplete Block design, each plant constituting a block.

Half-leaf comparisons (lesion counts transformed log $(x+12)$)

TvS (*a*) Treatment and standard preparations were applied on opposite halves of each leaf of a plant the treatment preparation occupying alternate sides of the leaves. Exps. 4 D and 5 D, eight plants per treatment-standard comparison, four comparisons; Exp. 3 A, six plants per comparison, six comparisons (thirty-six plants were considered for this arrangement). Regarding each leaf as a block of two half-leaf units each comparison was analysed removing the variation between leaves and between sides of leaves.

TvS (*b*) Treatment and standard preparations were applied on opposite half-leaves. Each treatment occupied the left side of both leaves of four plants and right side of both leaves of four plants in Exps. 4 D and 5 D. In Exp. 3 A six treatments were considered and six plants used for each comparison. Results were analysed as in *TvS* (*a*).

R.B.Cov. Treatment and standard preparations were applied to opposite halves of each leaf of a plant the treatment occurring on the same side of each leaf. Plants were grouped so that each plant of a group contained a different treatment preparation. Each group was regarded as a block and each plant as a replicated pair of treatment units together with a standard. The analysis was a Randomized Block design with covariance.

BIBD. Exps. 4 D and 5 D: four treatments were applied randomly on the four half-leaves of each plant. Two groups of fifteen plants were analysed by Randomized Block design. Exp. 3 A: all possible combinations of six treatments taken four at a time were used, each combination occupying the four half-leaves of a plant. Two groups of fifteen plants were analysed by Balanced Incomplete Block design of four treatments per block.

Table 4. *Efficiency of experiment designs: bean*

Exp. ...	5D		4A		3A	
	Variance	Efficiency	Variance	Efficiency	Variance	Efficiency
Whole-leaves						
TvS	0·041584	1·00	0·025248	1·00	0·038740	1·00
R.B.Cov.	0·039008	1·07	0·019256	1·32	0·022870	1·69
BIBD	0·010620	3·92	0·008034	3·14	0·016180	2·39
Half-leaves						
TvS (a)	0·015288	2·72	0·009104	2·80	0·021072	1·84
TvS (*b*)	0·010520	3·95	0·008736	2·92	0·019317	2·02
R.B.Cov.	0·017360	2·39	0·024816	1·06	0·015390	2·52
BIBD i	0·004841	8·59	0·003878	6·51	0·006470	5·99
BIBD ii	0·005606	7·42	0·003064	8·24	0·006020	6·44

Table 4 shows the resultant variances of the difference of two treatment means on a per-plant basis, and the efficiencies of the various designs relative to the treatment-standard whole-leaf design. As the treatment-standard design for both whole-leaves and half-leaves involves a series of separate comparisons of each treatment with the standard the pooled estimate of error from within each comparison has been used as the average error of the experiment, the variances shown in Table 4 being those of the difference of two treatment-standard differences. For designs involving the technique of covariance an average error for all treatment comparisons was obtained using Finney's (1946) method.

A marked superiority of half-leaf designs over those involving whole leaves is apparent, while of the whole-leaf designs considered, the Balanced Incomplete Block arrangement of all possible pairs of treatments appears to be the only one approaching the half-leaf designs in accuracy. The gain in accuracy by the use of designs involving covariance is small for whole-leaf units, whilst for half-leaf experiments there is no reliable increase. Designs involving the four half-leaf units of the plant as a block are approximately 3 times as accurate as the treatment-standard half-leaf designs.

Tobacco

Because the tobacco plant comprises leaves of differing ages and consequently differing susceptibilities, the plant itself is a block of rather heterogenous material. Homogeneity is maintained between halves of the same leaf however, and designs of the half-leaf within leaf type remain applicable. The efficiences of such designs involving six treatments on three groups of twenty-four plants taken from Exps. 2–4 of Table 2 are shown in Table 5. The following arrangements were considered:

TvS (*a*) Treatment and standard preparations were applied on opposite halves of each leaf of the plant the treatment occupying alternate sides of each leaf. Even numbers of plants for each comparison were taken to allow the treatment and standard preparation to occur equally on left and right sides of the leaves. Exps. 2–4 had four plants per comparison, with six comparisons. Each comparison was analysed as a two-way classification of leaves and sides.

TvS (*b*) Treatment and standard preparations were applied to opposite halves of each leaf, the treatment occupying left sides of all leaves of two plants and right sides of all leaves of two plants. Comparisons were analysed as above.

R.B.Cov. (*a*) Treatment and standard preparations were applied to opposite halves of each leaf of a plant, the treatment occupying the right side of each leaf. Plants were grouped randomly in pairs to form blocks. Each leaf of a block was assigned a different treatment, the treatments being assigned randomly to the leaves. Twelve groups of two plants were used, and results analysed as Randomized Blocks with covariance.

R.B.Cov. (*b*) Treatment and standard preparations were applied to opposite halves of each leaf of a plant, the treatment occupying the right side of each leaf. Four blocks of six plants were used each plant of a block being assigned a different treatment.

R.B.Cov. Leaf positions interchanged (*R.B. Cov.L.P.*). This design differed from R.B.Cov. (*a*) in the assigning of treatments to leaves of blocks. Treatments in the first group were assigned randomly and in each succeeding group a cyclic change was made to allow each treatment to occur an equal number of times on leaves of the same age. Variation between leaf position and groups was removed from the experimental error, the data being analysed by covariance. Twelve groups of two plants were used.

BIBD. The fifteen combinations of all possible pairs of six treatments taken two at a time were applied randomly to the fifteen leaves of five plants, each treatment being applied to a half-leaf. Groups of five plants were taken in each experiment. Balanced Incomplete Block design analysis was used.

All Possible Pairs. Leaf positions interchanged (*A.P.P. L.P.*). The fifteen possible pairs of six treatments were applied to fifteen leaves of five plants, the pairs being arranged so that all treatments occurred once on each plant. Three groups of five plants were taken, the treatments being cyclically interchanged from group to group to allow each treatment pair to occur once in each leaf position. The design

TABLE 5. *Efficiency of experiment designs: tobacco*

Exp. ...	3		2		4	
	Variance	Efficiency	Variance	Efficiency	Variance	Efficiency
TvS (*a*)	0·011304	1·00	0·012212	1·00	0·012132	1·00
TvS (*b*)	0·011756	0·96	0·013044	0·94	0·012540	0·97
R.B.Cov. (*a*)	0·008096	1·40	0·011616	1·05	0·011172	1·09
R.B.Cov. (*b*)	0·008624	1·31	0·010696	1·06	0·010872	1·12
R.B.Cov. L.P.	0·008700	1·30	0·010452	1·17	0·010192	1·19
BIBD	0·003455	3·27	0·002335	5·23	0·003820	3·18
A.P.P. L.P.	0·004115	2·75	0·002942	4·15	0·003163	3·84

was analysed as a two-way classification of plants and leaf positions. It is seen that all designs are at least as accurate as the treatment-standard design. The use of covariance technique, together with the removal of variation between leaves of different ages, results in improved accuracy. Covariance alone using leaf or plant as unit of randomization has a less marked effect. Designs involving all possible pairs in a Balanced Incomplete Block arrangement show a reliable gain in accuracy over other designs.

Nicotiana glutinosa

The variances of treatment comparisons for three half-leaf designs superimposed on two groups of twenty-four *N. glutinosa* plants from Exps. 1 and 2 of Table 2 are shown in Table 6. The experimental arrangements were as follows, all designs using six leaves of each plant and comparing six treatments.

T v S. Treatment and standard preparations were applied to opposite halves of each leaf of a plant, four plants being used for each treatment-standard comparison. Treatments were applied to the left and right sides of the leaves of each plant alternately. Six comparisons were used, each comparison being analysed as a two-way classification of leaves and sides.

R.B.Cov. (*a*). Treatment and standard preparations were applied to opposite half-leaves, the six treatments occurring in random position on the right sides of the leaves of each plant. Results were analysed as Randomized Block design with covariance, each plant being a block.

R.B.Cov. (*b*). Treatment and standard preparations were applied to opposite half-leaves, treatments occupying the right-hand side. Four blocks of six plants were used, each plant of a block being assigned a different treatment. Results were analysed by covariance as randomized blocks with removal of leaf positions.

Latin Square Covariance (*L.S.Cov.*). Treatment and standard preparations were applied to opposite half-leaves, the treatment occupying the right side of each leaf. Each treatment occurred once on each plant and occupied each leaf position once in each of four Latin squares. Results were analysed as a chain of four six-by-six Latin squares with removal of variation due to leaf position and plant.

TABLE 6. *Efficiency of experiment designs:* Nicotiana glutinosa

Exp. ...	1		2	
	Variance	Efficiency	Variance	Efficiency
TvS	0·007232	1·00	0·006252	1·00
R.B.Cov. (*a*)	0·003840	1·90	0·006028	1·04
R.B.Cov. (*b*)	0·003564	2·03	0·005108	1·22
L.S.Cov.	0·003404	2·12	0·005432	1·15

The use of covariance, together with removal of variation due to leaf age, again results in increased accuracy, while the randomized block with the plant as the unit of randomization is equally as accurate as the more complex Latin square arrangement.

DISCUSSION

Relative infectivities of virus preparations are determined experimentally by the mean number of local lesions produced per half-leaf, leaf or plant. Analysis of variance may be applied to the lesion numbers from which these means are obtained to ascertain whether the differences are real or due to variability of the leaves or plants rather than the preparations being tested. To overcome this variability of leaves or plants, preparations may be applied to leaves or half-leaves in a design which allows its effect to be reduced to a minimum.

Information obtained in the above experiments has been used to study the effect of plant and leaf variation on designs of comparative trials. A clearly linear relation holds between the mean standard deviation and mean lesion count. The same relation holds for bean, tobacco and *N. glutinosa* plants each inoculated with different viruses. The intercept on the m axis of the regression line of standard deviation of the mean gives the transformation necessary to render the standard deviation independent of the mean. For half-leaf experiments on bean, tobacco and *N. glutinosa* the most reliable transformation is log $(x+12)$ and for whole leaf experiments on bean log $(x+21)$ is most suitable.

The calculations have been based on means between 7 and 260 lesions per half-leaf so that no information is available for means outside this range. However, since most workers usually adjust their inocula to give lesion numbers of between 10 and 100 per half-leaf the above transformations will hold for most experiments of this nature. Use of the same transformation for all experiments eliminates any possible bias in favour of a particular comparison.

Although in most cases variation between the relative accuracies of any two designs appears large and dependent to some extent on the practical conditions of the experiment, general inferences may be drawn from the data.

The superiority of the half-leaf over whole-leaf inoculation technique first shown by Samuel & Bald (1933) is again apparent. In all half-leaf designs the use of the technique of analysis of covariance alone to remove the effect of leaf susceptibility results in little gain in accuracy over the treatment-standard comparison design. However, when used in conjunction with designs allowing the removal of both plant variation and that due to leaf age, reliable gains in accuracy are obtained. For treatment-standard comparisons the use of the plant as unit of randomization is for tobacco almost as accurate and for bean much more accurate than the use of the leaf. With randomized-block designs this is again apparent so that in these cases the simpler design, which is quicker to carry out experimentally is to be preferred. Designs of the Balanced Incomplete Block type show an even greater increase in accuracy when compared with the treatment-standard design on a per-plant basis. As these designs require no standard preparation the number of half-leaves available to the treatment preparations in any one experiment is thus doubled. This allows greater replication of the treatment material with subsequent reduction in variation.

It is apparent that experiments involving the treatment preparations inoculated on half-leaves in a Balanced Incomplete Block arrangement are the most accurate for determining the relative infectivities. While the incorporation of the standard half-leaf and the use of the Treatment × Standard design provides the most accurate treatment-standard comparison the accuracy of direct treatment comparisons tends to be reduced by the introduction of the standard.

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REVIEWS

An Outline of Developmental Physiology. By CHR. P. RAVEN, translated by L. DE RUITER. Pp. viii + 216 with 10 plates and 64 text-figures. London: Pergamon Press, Ltd. 1954. 17*s.* 6*d.*

This is an excellent translation of an admirable text-book of experimental embryology. It is a pity that, owing to the war, the original was written six years before it was published in Dutch, and that the English edition has not appeared for a further six years. Yet that this edition has been brought up to date so far as possible is shown by the fact that more than half of the references refer to publications which have appeared since the work was written, and more than a quarter since the Dutch edition was published.

The reviewer recommends this book warmly as, in his opinion, it provides the most satisfactory small text-book of experimental embryology for the use of undergraduates. Written by a distinguished contributor to the field, it is both authoritative and balanced. The style is very clear and the arrangement is coherent and logical. The author, by the careful choice of his material, avoids, on the one hand, including too much factual matter and, on the other hand, a too theoretical approach; faults from which other text-books of this subject suffer too often.

The title of the English edition is, perhaps, unfortunate; it deals almost entirely with that branch of experimental embryology which is often, and perhaps best, described as morphogenesis. There is no mention, for example, of the work of the Barcroft school on mammalian foetal physiology, nor would it be appropriate in such a book, yet it is undoubtedly 'developmental physiology'. More remarkably there is no mention of the work of Rothschild on the physiology of fertilization, which should have been included. Again, the dust-cover is quite misleading in calling the book 'a descriptive text-book of invertebrate and vertebrate embryology'.

The book is admirably produced; convenient in size, well printed and well illustrated. It contains a useful glossary of technical terms, a list of references and a good index. The price is very reasonable.

F. W. ROGERS BRAMBELL

Recent Developments in Cell Physiology. Ed. J. A. KITCHING. Pp. x + 206. London: Butterworths Scientific Publications. 1954. 40*s.*

The modern fashion of holding a symposium upon a topic of general interest has developed within the last twenty years. It is undoubtedly designed to counteract the sense of isolation which the needful specialization of research technique engenders in the conscientious worker and also to mitigate the evolutionary aberrations all too liable to arise within an isolated community narrowly inbred in its ideas. The contributions to these symposia with the discussion they stimulate frequently appear as a book.

The report of the Colston Symposium upon recent developments in cell physiology which was held in Bristol from 29 March to 1 April 1954 appears with most praiseworthy speed so that the contents still justify the promise implied in the title, as sufficient time has not yet elapsed for the majority of the communications to appear as contributions in the appropriate separate specialist journal. The editor, publisher, and Bristol University, acting through the Colston Society, accordingly deserve our sincere thanks.

The fifteen contributors were either resident in this country or were invited from Belgium and Denmark, so certain internationally eminent symposiasts are not represented. The deliberations of this Rump makes stimulating reading none the less. Though the range of material covered is wide, diffuseness is avoided since the topics for exposition and discussion on each day were deployed around some unifying theme. These were: cell permeability and active transport, synthesis of proteins and nucleic acids, the cell nucleus in differentiation, regeneration, cell cleavage and cell metabolism, and finally a contribution from the editor on suction in the suctoria. The level of treatment in the contributions varies from reviews salted with speculation to detailed exposition with relevant technical information of comparatively recent experimental work. The whole constitutes a most stimulating and valuable book.

The first topic considered was that of exchanges of material between cell and surround considered in general, with special emphasis on active transport by J. F. Danielli, in detail in respect to the crab (*Eriocheir*) gill by H. J. Koch, with regard to interpretation of membranes as sieves with the transfer problems of pore resistance by H. H. Ussing and finally a concise and elegant review of the ionic permeability of nerve membranes by R. D. Keynes.

The second topic was concerned with protein synthesis and the biochemistry and biology of nucleotide synthesis and content in both animal and plant material. It seems to the reviewer that this joint treatment is of the greatest value as workers in plant and animal material rarely seem aware of the possibilities and successes of research in both fields. The relationship between cellular oxidations and the synthesis of amino-acids and amides in plants ranging from yeasts to barley embryos was considered by E. W. Yemm. H. Klenow followed with an account of the biosynthesis of pentoses, and E. Hoff-Jørgensen gave an account of his elegant assay procedure for measuring the DNA content of biological material and the results from its application to sea-urchin, frog and chick gametes. The role of the nucleus in the control of enzymic activities was outlined by J. Brachet, together with a comparative study of nucleated and enucleate fragments of *Amoeba proteus* and the marine alga *Acetabularia*. C. H. Waddington reviewed the variety of experimental evidence concerning the existence and significant functioning of plasmagenes and concluded with a more detailed exposition of his already published theory of embryonic differentiation.

The cyto-physiological implications of regeneration processes in planarian worms was discussed by H. V. Brøndsted who succeeded in demonstrating a probable relation between his observations and the field and gradient phenomena of the experimental embryologist.

The final organization centre for discussion was cell-division and the energetic and metabolic subsidies it requires. Synchronous division both in protozoa (E. Zeuthen and O. Scherbaum) and bacteria (O. Maaløe and K. G. Lark) can be established in a culture by a routine of temperature shock, and this near simultaneity is of great value in metabolic studies. In bacteria DNA synthesis appears to have a different temperature coefficient from cell growth, and nuclear division is not followed immediately by cell division. The relative influence of environmental and genetic factors on the formation of protoperithecia in *Neurospora* was examined by M. Westergaard and H. Hirsch. They demonstrated a probable relationship between tyrosinase activity, with consequent melanin formation, and the differentiation of female sex organs. The control of cell-division by accumulation of some essential metabolite, the accumulation of which is initiated before the previous cleavage is completed, was argued persuasively by M. M. Swann. Experimental evidence in support of this hypothesis was obtained from the effect of carbon monoxide and ether upon the totally cleaving egg of the sea-urchin.

The editor, J. A. Kitching, completes the presentation of formal papers with a discussion on suction in the suctoria. These fascinating organisms appear to achieve this end by swelling. The editorial vigilance in proof correcting it perhaps understandably relaxed in this contribution; for example 'oxosmosis' seems a charming concept. Amongst the very few other misprints and omissions is might assist the reader to know that $c_{W(i)}$ on p. 34 refers to concentration, whereas $a_{W(0)}$ on page 36 refers to activity and that the distinction between these, if the comment on p. 37 is accepted, would seem excessively academic in the context. On p. 140 Dr Meryl Rose is spelled phonetically as is perhaps natural in the report of a discussion. The printing of some of the half-tone illustrations is not very satisfactory—the paper used does not seem to have been suitable. Finally a list of participants in the symposium is given, their collective portrait acts as frontispiece; there is no sign of any key whereby face and name may be correlated. There is no index, so intending seekers after knowledge will be constrained to read the entire work, which it is the aim of this reviewer to persuade them to do.

SYDNEY SMITH

Conservation par le Froid des Denreés d'Origine végétale. By ROGER ULRICH. Pp. 328. Paris: Ballière et fils. 1954. 2500 fr.

This book, one of a series of an Encyclopedia of Refrigeration, is chiefly devoted to the subject of cold storage of fruits and vegetables. The first eight chapters deal with the botanical and chemical structures of plant cells; the effect of humidity, temperature and atmosphere on the storage life of fruits and vegetables; common rots and breakdowns surveyed in general, and also in detail for the main kinds of stored produce. Special attention is given to apples, pears, plums, peaches and citrus fruit, with references to some twenty-four other fruit species. The chapter on vegetables is equally comprehensive. All these chapters are fully illustrated with photographs and diagrams from the research laboratories at the Station du Froid de Bellevue.

There is a short chapter on holding flowers, seeds and bulbs in cold storage, and another which collects together various manufactured products, from penicillin and vaccines, to fruit juices and alcoholic beverages, all of which are stored at low temperatures.

The chapter on quick freezing gives a summary of the processes necessary for fruits and vegetables, with an interesting table on the increased bacterial count of quick-frozen fruits on thawing and standing 24 hr. at room temperature. In the final chapter mention is made of the use of freeze drying and freeze concentration.

The bibliography at the end is extensive and shows, as the author explains in the introduction, the lack of French publications on the subject, and that most of the research has been published in this country and in the United States. References are also given to work from Trinidad, Capetown and Germany. It is a pity that there is no index in a volume of this nature which is essentially a reference book.

M. STURDY

CORRECTION

The price of *Bulletin* 42 of the Commonwealth Bureau of Pastures and Field Crops is 35*s*., not 15*s*. as stated on p. 539.